Corticocortical connections in man

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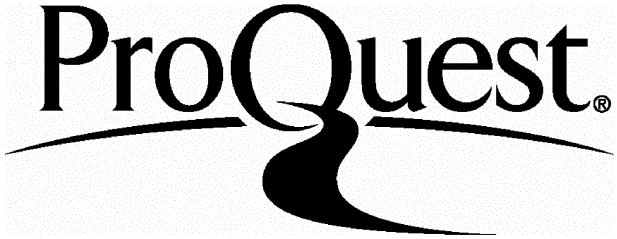

Abstract

Anatomical and electrophysiological evidence suggests that there is a dense network of intrinsic interneurones in the motor cortex. Many of these neurones appear to have an inhibitory action. The experiments described in this thesis examine the importance of a subset of these intracortical inhibitory neurones in motor control.

Using the relatively new technique of transcranial magnetic stimulation it is now possible to study the motor system in awake human subjects. This painless, non-invasive technique allows us to study the functioning of the motor cortex in humans both during rest and while making natural movements, something that has been impossible until recently. By employing a double pulse magnetic stimulation technique it is possible to study the efficacy of intracortical inhibitory actions. The experiments described in this thesis examine the role of the inhibitory system in normal subjects at rest and during voluntary contraction. The results are compared with those from two groups of patients and controls. The first group consisted of patients suffering from disorders of movement (myoclonus, dystonia and Parkinson's disease). The second group consisted of subjects who had suffered either temporary (normals with ischaemic blocks) or permanent (traumatic amputees) deafferentation.

The results suggest that the observed inhibition has an important role in the functioning of the motor cortex. In the conditions studied, when there are disorders of motor control there are also clear abnormalities in cortical inhibition. It appears that abnormalities in inhibition in the motor cortex may be caused by either intrinsic pathology (e.g. as seen in cortical myoclonus) or provoked by pathology elsewhere in the central nervous system (e.g. the basal ganglia in Parkinson's disease or focal dystonia). It is possible that the disordered cortical inhibition may contribute to some of the symptoms seen in these conditions.

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Publications

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Chapter 1

INTRODUCTION

The motor cortex- anatomy

Microscopic organisation of the cortex

Most of the cerebral cortex in man is neocortex (also termed isocortex). The microscopic structure of sections of the cortex stained for nerve cells has the following general organisation. Different areas of cortex have minor alterations in this general architecture. Six separate layers are seen:

I. *The molecular or zonal layer.* This layer is immediately below the pia and is rich in fibres but poor in cells. A few small nerve cells are present, the horizontal cells of Cajal. These cells have their longest axes running parallel to the cortical surface and form a dense tangentially running plexus.

II. *The external granular layer.* This layer is composed of densely packed small cells, some of which are a pyramidal type and some are round or star shaped. The apices of the pyramidal cells are directed towards the surface.

III. *The pyramidal layer.* This layer consists predominantly of medium sized pyramidal cells. The larger pyramidal cells are found in the deeper parts of this layer.

IV. *The internal granular layer.* This layer is mostly composed of small cells lying close together. These cells are equally split between star shaped cells and pyramidal cells. This layer also has abundant horizontally directed fibres.

V. *The ganglionic layer.* This layer is composed mainly of medium sized and large pyramidal cells. They have long apical dendrites which are directed towards the molecular layer. Also, they have abundant basal dendrites which run more or less horizontally.

VI. *The multiform layer.* This layer contains predominantly spindle-shaped cells. This layer can be subdivided into an outer part VIa and VIb. The latter of these parts fuses with the white matter at many places.

Cytoarchitectural subdivisions

Much of the anatomical study of the cortex has been performed in monkeys. Brodmann areas 4 and 6 contain the main motor areas of the frontal cortex. These areas are distinguished from other areas by their relatively poorly formed internal granular layer (IV). These motor regions are bounded by area 8 (frontal eye field)

anteriorly and by area 3a posteriorly which is part of the primary sensory cortex situated within the central sulcus. Area 4 differs from area 6 in having giant Betz cells. This distinction is relatively clear in the medial part of area 4 where the Betz cells project to the leg. However, in the more lateral arm area, the Betz cells are much smaller and it is more difficult to define the boundary.

It is now recognised that area 6 has three separate regions, an inferior and superior section on the lateral aspect of the hemisphere, and a mesial area. The superior and inferior areas are separated by the spur of the arcuate sulcus; the mesial and superior regions by their locations on different surfaces of the cortex. Mesial area 6 borders ventrally on the agranular cingulate cortex (areas 23 and 24) which is now thought to be an important motor region.

Area 4 (precentral cortex) is known as the primary motor area. Area 6 and regions of the cingulate cortex are known as the non-primary motor areas. Strick and colleagues have suggested that a non-primary motor area should be defined by two criteria: (1) presence of pyramidal neurones with axons terminating in the spinal cord, and (ii) corticocortical connections with the region of primary motor cortex which sends axons to the same spinal segments. On the basis of these definitions there are 6 subregions of non-primary motor cortex that are devoted to control of the arm (see Fig. 1.1). These are:

(1) the inferior limb of the caudal bank of the arcuate sulcus (the arcuate premotor area (APA)),

(2) the lateral edge of the superior precentral sulcus (SPcS), just anterior to the primary motor cortex proper,

(3) mesial area 6, in the region corresponding to the supplementary motor area (SMA), at about the same anterior extent as the genu of the arcuate sulcus on the lateral surface of the hemisphere,

(4) three small areas of cingulate cortex:

(a) a rostral cingulate motor area (CMAr) on the dorsal bank of the cingulate sulcus, anterior to the genu of the arcuate sulcus.

(b) a caudal area on the ventral bank of the sulcus (CMAv), and

(c) a second caudal area on the dorsal bank of the sulcus (CMAd).

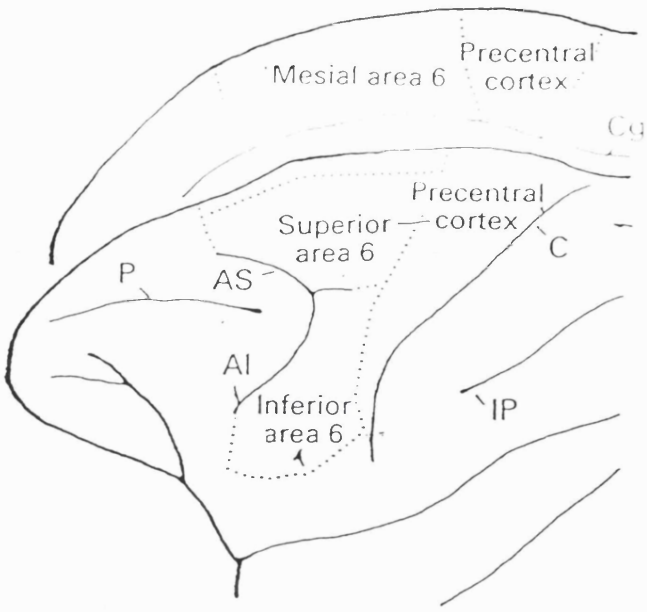
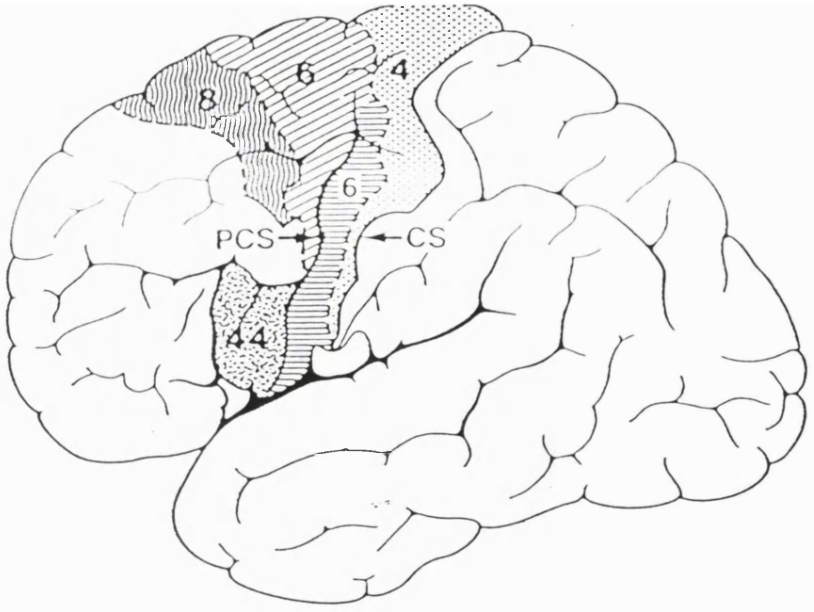


Fig. 1.1. Dorsolateral and mesial views of the brain showing the general subdivisions of the agranular frontal cortex according to Matelli and Luppino. P, principal sulcus; AS, superior limb of the arcuate sulcus; AL, inferior limb of the arcuate sulcus; C, central sulcus; IP, intraparietal sulcus; Cg, cingulate sulcus.

The output from these areas represents about 60% of the total frontal lobe projection to the spinal cord. Each of these areas has a connection to the arm area of the primary motor cortex.

Fulton's (1935) original concept delineated the human premotor area as the frontal agranular cortex (area 6) rostral to the primary motor cortex (area 4). This area shows enlargement in humans where it is five times larger than area 4 as compared to the 1:1 relationship in the macaque (Bailey and von Bonin, 1951). Fig. 1.2 shows the distribution of Brodmann's areas 4, 6, 8, 44 on the precentral and frontal gyri. When the human inferior precentral sulcus is taken to represent the homologue of the arcuate sulcus in monkeys, the ventral compartment of area 6 (PMVc in Fig. 1.2) covering the banks of the precentral sulcus and the anterior part of the precentral gyrus corresponds to the monkey's arcuate premotor area. The largest part (PMCd) lies on the superior and middle gyrus.



dorsal compartment of area 6 on superior and middle frontal gyrus (PMC d)

ventral compartment of area 6 on precentral gyrus (PMC v)

PCS precentral sulcus

CS central sulcus

Fig. 1.2 Distribution of Brodmann's agranular and dysgranular cytoarchitectonic areas 4, 6, 8, and 44 on the precentral and frontal gyri. Different cytoarchitectonic areas and subdivisions of area 6 (into a ventral compartment (PMCv) and a dorsal part (PMCd)) are shown.

Specialisation of the primary motor cortex

The motor cortex has several characteristic differences from other regions. The density of neuronal cells is at its lowest in the motor cortex. The cortex is thicker in this region than in other areas. Rockel *et al.* (1980) report that in man the motor cortex is 1.6 times thicker than primary visual cortex. Due to the thickness of the cortex and the relatively low cell count there are large areas available in which to form synaptic connections.

There are two basic cell types in the motor cortex: pyramidal and nonpyramidal cells (Ramon y Cajal, 1911).

Pyramidal cells

The pyramidal cells form the main output system of the motor cortex and another characteristic feature of this region of cortex is the increased number of pyramidal cells in layers III and V and an internal granular layer that is relatively indistinct. Lamina V has the lowest packing density of cells of all the layers except lamina I (Sloper *et al.* 1979). There is a great range of pyramidal cell sizes, with the

giant Betz cells being the largest. The soma of these cells have diameters of up to 120 μm (Meyer, 1987). It has been estimated that there are approximately 20-25 cells per 100 μm^3 within lamina V (Humphrey and Corrie, 1978), and that a cylinder of baboon's motor cortex 1 mm^2 in area at the surface would contain approximately 90 large and 18 000 small pyramidal cells. The large Betz cells are seen near the superficial margin of layer V and form a tortuous line in the upper margin of this layer. They are often found in groups of about 2 to 4. (Gatter and Powell, 1978)

The dendrites of the pyramidal cells are covered with spines and receive massive synaptic inputs. Cragg (1975) estimated that there may be as many as 60 000 synapses upon a single pyramidal cell. In the motor cortex pyramidal cells are seen in clusters of approximately three to five neurons (Jones and Wise, 1977). The apical dendrites of these clustered cells ascend to the most superficial layers of cortex in close association (Feldman, 1984). Synaptic connections between these closely associated cells would provide a rich network for communication between clustered neurons (Porter and Lemon, 1993). There are clear morphological differences between pyramidal cells in the different cortical layers. The axon collaterals of lamina V neurones have a restricted distribution and terminate within laminae V and VI, whereas lamina III axon collaterals are extensive and provide synaptic contact with all laminae of the cortex (Ghosh *et al.* 1988).

Non-pyramidal cells

The most numerous non-pyramidal cells in the motor cortex are the large aspinous stellate, or basket cells (Sloper *et al.* 1979; Meyer, 1987). These cells are mainly found in cortical layers III, IV and V. Almost exclusively the targets of the basket cells are within the cortex. They make inhibitory GABAergic connections with pyramidal neurones and are a prime candidate for inhibitory effects exerted by collaterals of corticofugal neurones (Jones 1983). The axons of the basket cells are myelinated and lie in a horizontal orientation. The pyramidal cells and the basket cells make up by far the majority of the cells within the motor cortex. However, there are also smaller neurogliaform cells which have a much more localised connectivity (Porter and Lemon, 1993), and some chandelier cells which can be seen in cortical layer II. Recently Deuchars and Thomson (1995) have shown in slices of rat somatosensory cortex that there are interneurons that can produce short latency

(rise time <5ms) IPSPs in identified pyramidal cells. They propose that these effects are brought about via $GABA_A$ receptors. It may be that similar arrangements exist in the motor cortex.

Intrinsic connections

Intercolumnar connections

One of the prominent features of motor cortical neurons is their long horizontal branches. Pyramidal neurons in layer V can have horizontal axon collaterals that travel for distances of up to 2 mm within layers V and VI (Landry *et al.*, 1980; Ghosh *et al.*, 1988). The axon collaterals of pyramidal cells in layers II-III also project over substantial distances and often form clusters of axon terminals ((Landry *et al.*, 1980; Keller and Asanuma, 1993). The targets of these collaterals are both neurons in close proximity and also distant neurones. They can provide both excitatory and inhibitory input to distant pyramidal neurones (Keller, 1993). Huntley and Jones (1991) using intracortical microstimulation and horseradish peroxide labelling demonstrated extensive, horizontally orientated collaterals which provided input to many different forelimb movement representations.

Intracolumnar connections

Pyramidal neurons in the motor cortex also have local collaterals (Feldman, 1984). There are usually one to three collaterals from the proximal portion of the main axon which project towards the pial surface (Keller *et al.*, 1990; Keller and Asanuma, 1993). These collaterals form clusters of axon terminals in the immediate vicinity of the parent soma and the proximal portion of the apical dendrite. Pyramidal neurons in the superficial layers of cortex have short axon collaterals that terminate in layer V immediately below their parent soma. Many of these collaterals form synapses with dendritic spines of pyramidal cells (Keller and Asanuma, 1993). The majority of synaptic inputs to both pyramidal and non-pyramidal cells comes from local intrinsic sources (Gatter and Powell, 1978). Much of this input is thought to be inhibitory in nature (Jones, 1983).

Fibre connections of the cerebral motor areas of cortex

Corticofugal projections

The most studied of the efferent fibre projections is the pyramidal tract. It has corticospinal and corticobulbar fibres which arise in various cortical areas most of which are in the pre- and postcentral gyrus. This system is discussed in more detail later in this section. First a summary of some of the other major connections is given.

In primates there are direct projections to the cranial motor nuclei. These consist of bilateral, but predominantly contralateral, projections to the facial nucleus (Jenny and Saper, 1987), and bilateral projections to the trigeminal and hypoglossal nuclei (Kuypers, 1958a,b, 1981; Iwatsubo *et al.*, 1990). These direct projections arise from the caudal part of the precentral gyrus. In addition there are direct projections to the VL nucleus of the thalamus and both the parvocellular and magnocellular red nucleus with projections to the former being the most numerous (Catsman-Berrevoets *et al.*, 1979). Kuypers (1981) showed that these projections were almost exclusively derived from the precentral gyrus (areas 4 and lateral area 6).

Corticoreticular projections arise from wide areas of the cortex (Keizer and Kuypers, 1989) including the rostral parts of the precentral gyrus (area 6). These fibres are distributed ipsilaterally to the pontine and bilaterally to the medullary reticular formation. Their targets include the raphe nuclei and the reticular nuclei which give rise to the reticulospinal tracts.

Area 4 also has corticocortical connections to parietal areas 2 and 5. There are also reciprocal connections with the SMA and premotor cortex. There are also important outputs to the basal ganglia, pontine nuclei, and reticular formation. Non-primary areas of motor cortex also have indirect connections with the spinal cord. The SMA has efferents that project to the red nucleus and areas of the reticular formation. The premotor area projects to the medial tegmental region of the brainstem. Also, there are projections to the pons (and thence to the cerebellum) and basal ganglia.

There are also abundant topically organised fibres that pass to the thalamus forming the corticothalamic tract. These fibres pass largely to the thalamic nucleus from which the cortical area emitting them receives its thalamic afferents.

Many of these efferent fibre systems originate in the central regions of cortex (areas 4, 3, 1, 2, 6) and any one region of cortex may project to multiple subcortical sites (to the thalamus, brainstem, basal ganglia etc.)

The pyramidal tract

The major outflow from the primary motor cortex is via the corticospinal tract. Those corticospinal fibres that pass through the medullary pyramid are known as the pyramidal tract. In the macaque monkey the corticospinal tract is made up by the axons of pyramidal cells in layer V of the cortex (Coulter, Ewing and Carter, 1976). The giant Betz cells in the primary motor cortex give rise to the largest (11-20 μm) and most rapidly conducting fibres in the corticospinal tract. However, only 3-4% of the pyramidal tract is accounted for by these very large fibres. The vast majority (90%) of fibres are small (1-4 μm) and poorly myelinated.

Many of the pyramidal fibres originate from areas outside the primary motor cortex. Pyramidal fibres have been shown to originate in cortical areas 3, 1, 2, 5 (Levin and Bradford, 1938), 6 (Kuypers, 1987) as well as from area 4. Ablation of area 4 leads to degeneration of approximately 30-40% of the pyramidal tract (Lassek, 1942). Data from human subjects is quite sparse. Nathan and Smith (1955) concluded that most of the cells of origin of the human corticospinal tract were situated in the precentral gyrus, mainly its upper two thirds. In a single case, following ablation of the precentral gyrus, it was estimated that 60% of all medullary pyramid fibres arose from area 4 (Jane, Yashon, DeMeyer and Bucy, 1967).

The non-primary motor areas are located in parts of cytoarchitectonic area 6 on the lateral surface and medial wall of the hemisphere (Kuypers, 1981), as well as in subfields of areas 23 and 24 in the cingulate sulcus (Dum and Strick, 1991). Dum and Strick (1991) recently showed in the monkey that corticospinal projections to the cervical spinal cord originated in the primary motor cortex and 6 non-primary motor areas in the frontal cortex. All these regions also have a direct input to the primary motor cortex. The total number of corticospinal neurons in the arm representation of the non-primary motor areas equals or exceeds the number from the arm

representation of the primary motor cortex (Dum and Strick, 1991). The functional contribution of the corticospinal projections from the non-primary motor areas remains unclear. However, results from preliminary studies (Brinkman, 1982) suggest that the corticospinal projections from the dorsal cingulate motor area (CMAd), ventral cingulate motor area (CMAv), and SMA terminate most heavily in the intermediate zone of the spinal cord. The intermediate zone of the spinal cord contains many interneurons that project to motoneurons and thus the limited evidence suggests that the corticospinal projection from the non-primary motor areas has access to spinal cord mechanisms concerned with motor output.

In primates, area 4 and the inferior portion of area 6 give rise to the corticospinal projections to the dorsal and lateral part of the contralateral spinal intermediate zone and the spinal motoneurones (Kuypers, 1987). These pathways makes intimate contact with spinal motoneurones of both distal and proximal muscles in the grey matter of the spinal cord. Fibres which make direct contact tend to be of large diameter and rapidly conducting, and originate in the bank of the central sulcus. Axon collaterals of corticomotoneuronal cells make direct monosynaptic contacts with motoneurones located in several different segments and contact the spinal motoneurones of several different muscles (Shinoda, Zarzecki and Asanuma, 1979). However, the large numbers of direct monosynaptic contacts formed within the spinal cord by corticospinal tract fibres has led to the concept that it is concerned mainly with control of the hand, and is important in the performance of fractionated finger movements. In contrast, the ventromedial pathways descending from the brainstem are thought to be concerned with the control of axial muscles (Lawrence and Kuypers, 1968).

Afferent connections of cortical motor areas

Evidence now suggests that the afferent input to the motor cortex is arranged in a highly specific manner, with dense regions of terminations and areas where there are few terminations (Shinoda and Kakei, 1989).

Thalamocortical afferents

Afferents from the thalamus have been shown to reach extensive regions of the neocortex and are known to terminate in all layers of the cortex (Strick and Sterling, 1974; Sloper and Powell, 1979). These terminations are densest in layers II

and at the III/IV border. There is also a second dense band of terminations in the deeper part of layer V. Most of these afferents terminate on dendritic spines of pyramidal neurones and make type1 (asymmetric) contacts which are presumed to be excitatory (Porter and Lemon, 1993).

Particular cortical areas receive their input from specific thalamic regions. Sensory afferents from the face terminate in VPM (ventroposterior medial) and from the body in VPLc (ventroposterior lateral pars caudalis)

Each large body part is represented as a kind of curved lamella. Lateral lamellae tend to be more highly curved than medial ones and the mapping is continuous (Jones and Friedman, 1982). Within the fields of termination of these afferents a core is formed by afferents from cutaneous receptors. This core projects to cortical areas 3b and 1. On the outer side of this cutaneous core there is a thin region that responds to stimulation of deep tissue (Friedman and Jones, 1981). This region of deep tissue receptors projects to cortical areas 3a and 2.

The precentral gyrus and central sulcus receive predominant input from different subdivisions of the ventrolateral thalamus. VPLo (ventroposterior lateral pars oralis) provides the most substantial input to a portion of the hand representation on the gyrus. The VPLo receives input from rostral deep cerebellar nuclei. VLo (ventralis lateralis pars oralis) provides the most substantial input to a portion of the hand representation in the sulcus (Holsapple, Preston and Strick, 1991) and also projects to the SMA. This region (Vlo) receives input from the globus pallidus. Area X projects to APA and receives input from the caudal cerebellar nuclei. Thus each thalamocortical pathway is associated with a distinct subcortical input (Strick, 1985). Some spinothalamic afferents end in VPLo (ventroposterior lateral pars oralis) which, as stated above, has a direct input to primary motor cortex. There is some evidence to suggest that the projection sites of the thalamus may not be as separate and clear cut as described above. For example, there is evidence that the cortical projection sites of the dentate nucleus include both the primary motor cortex and the APA (Orioli and Strick, 1989).

Corticocortical afferents

The majority of corticocortical afferents are local (Ghosh *et al.*, 1987) and arise from pyramidal cells in laminae II and III. These afferents terminate in all

layers of cortex and make contact with the dendritic spines of pyramidal cells and the dendritic shafts of non-pyramidal cells (Sloper and Powell, 1979). Apart from the local corticocortical afferents there are important interconnections between the six sub-areas of motor cortex.

Callosal afferents

The callosal inputs to the motor cortex arise from pyramidal cells in the deeper part of lamina III of the homotopic part of area 4 of the opposite hemisphere. They terminate in layers I through III, and almost exclusively on dendritic spines (Lund and Lund, 1970; Sloper and Powell, 1979). There are fewer callosal connections between hand regions of motor cortex than between regions controlling the arm and trunk (Jenny, 1979; Pappas and Strick, 1981; Gould *et al.*, 1986).

Effect of lesions of cortical motor areas

Precentral gyrus

Damage of the precentral gyrus in humans represents the most elementary motor disturbance. Destruction of the primary motor cortex or its descending fibres is associated with contralateral weakness. Spasticity is rare if the lesion is small and restricted to primary motor cortex. On recovery from the acute lesion the paralysis resolves first and the recovery is best seen in proximal muscles. Weakness of distal musculature and loss of fractionated finger movements are the remaining symptoms. These clinical observations are in keeping with the results seen in non-human primates from lesion studies and with what can be inferred from single unit recordings (Evarts, 1981). Recently, Hoffman and Strick (1995) showed that in the monkey lesioning of the primary motor cortex caused marked changes in movement kinematics and EMG activity patterns in a step tracking task. All movements were performed more slowly and became less smooth. Suppression of antagonist activity at movement onset was reduced or abolished.

The symptoms seen when there is damage to this region are in keeping with what would be expected. If there is damage or destruction of the output cells of the motor cortex then it is to be expected that there would be weakness or paralysis. Also, descending inhibitory influences would be lost which would lead to spinal cord disinhibition and exaggerated spinal cord reflexes. Many of the symptoms seen

with precentral gyrus damage are common to those seen following damage to the pyramidal tract.

Damage to the primary motor cortex can also lead to symptoms that are not evident after damage to the pyramidal tract. These symptoms can be classified as "irritative symptoms". The most striking of these is epilepsy, characterised in this cortical region by motor seizures. These symptoms are most likely due to damage of intrinsic connections within the motor cortex leading to a disinhibition of the ouput cells.

Supplementary motor cortex

There is a great amount of information regarding the important role of the SMA in movement initiation and preparation for movement. In the monkey, Tanji (1980) demonstrated that neurons in the SMA demonstrated instruction-induced changes of activity in the period intervening and instruction and a triggered response Even before this area was designated by Penfield and Welch (1949) there were reports that showed that bilateral medial frontal lobe damage may lead to a persistent complete akinesia and mutism (Critchley,1930). Talairach and Bancaud (1966) confirmed these reports by making well-defined excisions of SMA and cingulate cortex. Patients with unilateral operations initially showed severe akinesia, mainly on the contralateral side, associated with reduction of speech and emotional facial expression. Erickson and Woolsey (1951) observed a weak and short-lived grasp reflex after unilateral involvement of the SMA. This finding was later confirmed by Penfield and Jasper (1954) who also showed that even one year after the lesion there was still some slowness of movement, especially when the movements were alternating in nature. Subjects with unilateral lesions usually show some degree of recovery while those with bilateral lesions show very poor, if any, recovery. This is thought to be due to the fact that there are bilateral projections to subcortical target zones.

The symptoms seen after damage to the SMA are again in keeping with what would be expected with the knowledge of the SMAs role in motor control. The akinesia and mutism seen after lesions of the SMA fit with the well known role of the SMA in the planning and initiation of a movement, especially when the movements are more complex.

Premotor cortex(PMC)

Most of the disturbances following lesions of the PMC are nonspecific, such as clumsiness, loss of smoothness, loss of characteristic speed (Fulton, 1935; Kleist, 1911), or motor preservation and hemi-inattention (Laplane and Degos, 1983).

A special form of limb-kinetic apraxia was described in patients with unilateral PMC damage along with a mild, transient proximal weakness (Freund and Hummelsheim, 1985). This apraxia affects the coordination of the two arms or legs that is required during such tasks as making a windmill movement with the arms or a pedaling movement with the legs. Patients with these types of lesions have also shown pronounced deficits in sensory conditional motor learning (Halsband *et al.*, 1993).

In all of the discussions regarding the "appropriateness" of the symptoms following lesions of different cortical regions it is difficult to predict what might be expected as much of the knowledge regarding the functional role of the region has been gained through lesion studies. However, the finding of disturbed coordination between the eyes and the limbs, between different limbs and different muscular groups within a limb are consistent with a role for the PMC in temporal control of movement (Freund and Hummelsheim, 1985).

Effect of lesions of the pyramidal tract

Macaque monkey

From studies examining the effect of lesions of the pyramidal tract the following general conclusions have been made:

1. There is a permanent deterioration in the ability to make fine, independent movements of the digits. Lawrence and Kuypers (1968) found that monkeys were unable to retrieve small food particles from a modified Klüver board.
2. There is a permanent loss of contactual hand-orientating responses (Denny-Brown, 1966).
3. No long-term effects on axial/postural systems have been reported. Lawrence and Kuypers (1968) found that pyramidotomized monkeys could sit, stand, walk, run, and climb normally.

4. The severity of the deficit following lesioning depends upon the species. Paresis of the arm and loss of relatively independent finger movements (RIFM) were more marked in chimpanzee than in the macaque monkey (Tower, 1940).
5. The degree of recovery following a pyramidal lesion is dependent upon several factors such as (1) the size of the lesion, (2) duration and nature of postoperative period, and (3) the age of the animal (Hepp-Reymond, 1988).

Man

It is rare in man to find pathology confined to the medullary pyramids or pyramidal tracts (Phillips and Porter, 1977). These authors and others (see Wiesendanger 1969, 1981) conclude from the limited cases that damage to the pyramidal tracts produces some deficit of individual finger movements but does not result in marked spasticity. Bucy *et al.* (1964) report the case of a patient suffering from hemiballismus in whom the pyramidal fibres were sectioned unilaterally at the level of the cerebral peduncle. The operation resulted in an immediate cessation of involuntary movements and a flacid hemiplegia. The hemiplegia was followed by a period of improvement in strength and coordination which plateaued after about seven months. At this point the patient was able to perform fine, individual movements of the fingers that were only slightly less well executed than those on the unaffected right side. There was only mild spasticity and a weak Babinski sign. It was later revealed at post mortem that only 17% of fibres in the right medullary pyramid survived the section. It was also thought that many of these remaining fibres were from the parietal region. They attributed the good recovery of this patient mainly to the existence of indirect or extrapyramidal descending pathways.

Pure and complete hemiplegia has been reported following a vascular lesion in the medulla which produced a unilateral infarct restricted to the pyramidal tract (Ropper *et al.*, 1979). In this patient recovery was almost complete after a period of several years. Following focal ischaemic attacks, such as lacunar stroke affecting the internal capsule, skilled hand movements can be lost (Fries *et al.*, 1990). These patients often show rapid recovery. The mechanism behind this recovery is uncertain but there are at least three possible factors; (1) function may involve pathways other than the pyramidal tract, (2) the ipsilateral anterior corticospinal tract is well developed in man and it may be that this tract plays a role in recovery from insult (3)

it is now recognised that there is potential for plasticity within the central nervous system and it is possible that functional reorganisation is behind some of the improvements in function that can be seen following an infarct.

The primary motor cortex- Physiology

Pyramidal cells in the motor cortex are often seen in clusters of three to five cells. Direct confirmation that neighboring corticomotoneuronal (CM) cells do have similar muscle fields was provided by spike triggered averages of CM cells that were recorded simultaneously or successively (Cheney and Fetz, 1985). However, clear differences in the muscle fields of neighbouring CM cells projecting to the hand muscles can also be seen (Lemon *et al.*, 1987). Within a column of neurons different cells may be involved with different aspects of the movement. Kalaska *et al.* (1989), when examining populations of neurons, demonstrated that within a vertical array of neurons in the motor cortex, cells in layer V encode both the dynamic aspects and direction of movement, whereas cells in the superficial layers are concerned only with movement direction.

Using the technique of cross-correlation it has been shown that synaptic connections are much more common between neighboring neurones than those that are far apart (Renaud and Kelly, 1974: Allum *et al.*, 1982: Kwan *et al.*, 1987). Ghosh *et al.* (1988) demonstrated a presumed excitatory synaptic contact between axon collaterals of a slow pyramidal tract neuron (PTN) and the basal dendrites of a fast PTN. This contact is evidence of an excitatory coupling between the two cells. When recording from neighboring cells Smith (cited in Fetz *et al.*, 1990) found that of the cells that showed evidence of significant cross-correlation 90 per cent had findings that were consistent with a common excitatory input. The remaining ten per cent showed evidence of serial effects. This was split equally between neurones receiving either an excitatory or inhibitory input. It appears as if common excitatory inputs are most often seen in cells with similar task related patterns of activity.

Many corticomotoneuronal cells increase their firing rate in proportion to the force, or rate of rise of force, applied by the muscles employed in a particular task (Cheney and Fetz, 1980). Cheney and Fetz (1980) demonstrated that the increase in cortical cell discharge with force was greater for tasks that involved wrist extension rather than wrist flexion. The authors considered this finding consistent with the fact

that EPSPs were larger in wrist extensors than in wrist flexor motoneurones following cortical stimulation (Clough, Kernell and Phillips, 1968). The magnitude of post spike facilitation is greater for distal muscles than for proximal muscles (Cheney and Fetz, 1985; Lemon, Mantel and Muir, 1986; see below) and this was considered consistent with the finding of larger EPSPs in distal muscles than in proximal ones.

The type of task is very important in determining the type of cell discharge seen. Cheney and Fetz (1980) recorded the activity of a large number of corticomotoneuronal cells in monkeys while they were making two types of ramp and hold wrist responses. Depending upon the firing pattern all cells could be classified into four types: phasic-tonic (59%), tonic (28%), phasic-ramp (8%), or ramp (5%). All the cells were active during the static hold period; 'tonic' cells discharged at a constant rate, while 'ramp' cells showed steadily increasing discharge during the hold period. During the dynamic phase of the response (during the torque ramp) the 'phasic' cells exhibited an additional peak of activity which exceeded the final tonic level associated with the hold period. None of the discharge patterns of these cells was the same as the EMG profile and therefore it appears as if the corticomotoneuronal input is specific even within a task.

Muir and Lemon (1983) demonstrated a clear dissociation between the amount of corticomotoneuronal discharge of a particular cell and activity in the target muscle. They examined the activity of cells during the performance of power and precision grips in monkeys. Each of the cells discharged at higher frequencies during the precision grip task even though that in most cases the cell's target muscle was more active during the power grip. The conclusion from these experiments is that the firing pattern of corticomotoneuronal cells is task dependent.

These dissociation's between cortical neurone firing and target muscle activation suggest that there might be separate sub-populations in cortical neurones that are involved in different types of task. The timing of cortical cell discharge, with respect to the onset of EMG, also varies with the type of task being undertaken. Phasic cell activity tends to occur before the onset of EMG target muscle activity whereas tonic discharge tends to begin after the onset of EMG activity (Cheney and Fetz, 1980). Most of these studies have concentrated on large fast conducting

corticospinal fibres and Evarts (1965) showed that these types of neurones are important in the phasic elements of movement control. Changes in force, or the rate of change of force, produces the greatest change in discharge frequency of pyramidal neurones, with direction and amplitude of movement resulting in smaller changes (Evarts, 1969; Evarts, Fromm, Kroller and Jennings, 1983). Large and small pyramidal neurones have different characteristics. Large pyramidal neurones tend to be silent at rest, have relatively higher recruitment thresholds and discharge phasically at lower frequencies, while in contrast, smaller ones have lower recruitment thresholds and tend to discharge tonically at higher frequencies (Evarts *et al.*, 1983).

The output map of the primary motor cortex

The experiments of Fritsch and Hitzig (1870) were the basis of modern ideas regarding localisation of function within the motor cortex. They observed that galvanic stimulation (direct current) of different regions of the dog's cerebral cortex produced movement of different parts of the contralateral musculature. Ferrier (1876) extended these observations using faradic stimulation (alternating current) of the monkey motor cortex. Both of these types of stimulation do not evoke natural movement and the maps produced should be considered as "output maps".

Electrical stimulation of the motor cortex has been the most commonly used technique for studying the output organisation of the motor cortex. Phillips and his colleagues have shown that the output cells of layer V are directly excited by anodal stimulation of the cortical surface with brief pulses (Hern *et al.*, 1962; Phillips and Porter, 1977). However, this is only true for pyramidal tract neurons (PTN) whose long axis is orthogonal to the cortical surface. Stronger shocks are required to excite the many PTNs located in the anterior bank of the central sulcus which may be aligned tangentially to the cortical surface.

Using surface stimulation the somatotopy of the motor cortex is evident. Muscles can be activated from particular sites and some can be activated from several sites. This finding is particularly striking for movements of the digits (Penfield and Bouldry, 1937: Craggs and Rushton, 1976: Kwan *et al.*, 1978: Sessle and Wiesendanger, 1982: Humphrey, 1986: Donoghue *et al.*, 1992). Considerations of stimulus parameters (e.g. stimulus strength, single/multiple stimuli) are important

factors in determining the outcome of cortical stimulation. Even employing small stimuli, the spread of the stimulus makes interpretation of the results difficult. However, most motoneurones of distal musculature appear to receive convergent input from discrete "colonies" of motor cortex cells. These colonies can be distributed over several square millimeters of the cortical surface (Landgren, Phillips and Porter, 1962). Also, some muscles receive input from more than one area of cortex (Jankowska, Padel and Tanaka, 1975a).

The technique of intracortical microstimulation (ICMS) was developed by Asanuma and Sakata (1967) in response for a need to develop a more localised stimulating technique that was able to access cells buried deep in the anterior wall of the central sulcus that represented much of the distal musculature. This technique allows very small, well localised (stimuli used in this technique do not spread for more than a few millimeters) stimuli to be applied to the cortex. This form of stimulation may excite cortical cells by direct current spread to cell bodies (Stoney, Thompson and Asanuma, 1968; Cheney and Fetz, 1985), direct stimulation of axons (Asanuma, Arnold and Zarecki, 1976) or by indirect, transynaptic activation (Stoney, Thompson and Asanuma, 1968; Jankowska, Padel and Tanaka, 1975b). All of these mechanisms of stimulation can contribute to corticomotoneurone excitation (Ranck, 1975) and when repetitive stimulation is used, transynaptic activation becomes increasingly important. Often, the latency values seen following ICMS are approximately 1 ms longer than those obtained with spike triggered averaging and this extra time is thought to be related to the time taken for synaptic activation of pyramidal neurones following ICMS. Using ICMS the lowest threshold loci for stimulation are located in area 4 (Kwan, MacKay, Murphy and Wong, 1978). The threshold for eliciting movements is lowest when the stimuli are applied to layers II, III, VI and especially layer V (Kwan *et al.*, 1978).

The question of how the final output of the cortex to a particular muscle is represented in the cortex has been the subject of much debate. Landgren, Phillips and Porter (1962a) recorded motoneurone EPSPs after surface anodal stimulation and showed that the cortical cells projecting to a single muscle were widely spaced. There were discrete "colonies" of cortical cells that projected to individual muscles or small groups of muscles (Asanuma and Sakata, 1967). It was later suggested by

Asanuma and Rosen (1973) that small columns of cortical cells of about 1 mm in diameter project to single muscles. These columns of cortical cells have some degree of overlap. Kwan *et al.* (1978), using EMG recordings, found that the output cells of the motor cortex are organised radially in clusters, with cortical zones controlling movement of a proximal joint, partly encircling a more distal joint. Distal muscles had lower thresholds for excitation, and the greatest number of low threshold loci. These were obtained in the central region of area 4. For more proximal muscles the threshold becomes progressively higher from wrist to shoulder. Also, the EPSPs are larger for distal muscles of both the upper (Clough, Kernell and Phillips, 1968) and lower limbs (Jankowska, Padel and Tanaka, 1975b). This preferential accessibility of distal muscles is accompanied by a more limited spread of excitable sites. The excitable loci for more proximal muscles extend over a greater cortical area but are of a higher threshold (Phillips and Porter, 1964; Kwan, MacKay, Murphy and Wong, 1978).

The limitations of extrapolating the results from surface stimulation to functional considerations has been emphasised by several different groups (Phillips, 1987; Lemon, 1988). A different approach to this problem has been by using the technique of spike triggered averaging (STA). This technique investigates the activity of a group of cortical cells in relation to muscle activity (Fetz and Cheney, 1980; Muir and Lemon, 1983). When the spike triggered average of rectified EMG activity is examined the facilitatory effect of a corticomotoneuronal cell on its target muscle is seen as a transient increase in muscle activity following the cell discharge. There is a large amount of evidence that this post spike facilitation reflects monosynaptic, corticomotoneuronal activation of motoneurones (Fetz and Cheney, 1980; Lemon, Mantel and Muir, 1986). This technique probably gives a more realistic indication of a cells function in a natural movement and it is possible to obtain a "functional map" instead of the "connection map" obtained with surface stimulation and ICMS. This technique can be used in awake monkeys and so the function of the corticospinal projection can be examined during a variety of natural tasks. Using this technique it has been shown that single CM cells project more selectively to intrinsic hand muscle motoneurones than do cells controlling wrist

muscles. This finding suggests that there is less divergence of projections to distal musculature (Cheney and Fetz, 1985; Lemon, Mantel and Muir, 1986).

Following the discharge of a pyramidal neurone any associated EMG activity will be time-locked to the discharge and will be evident as an increase in the background EMG activity (post spike facilitation). In monkeys, Cheney and Fetz (1985) showed that the interval between cortical cell discharge and the onset of EMG activity in the forelimb muscles was 3.8-9.9 ms (mean 6.7 ms) during wrist movements. Lemon *et al.* (1986) reported a value of 4.1-15 ms (mean 9.8 ms) for forearm and intrinsic hand muscles during precise finger movements. There is greater facilitation of muscle activity when using ICMS than when using spike triggered averaging and this probably reflects a more synchronous activation of cells or a greater activation of cells. The technique of spike triggered averaging confirmed previous findings of cortical organisation i.e. cortical cells can project to the motoneurones of more than one muscle and conversely, that any one muscle may receive inputs from many cortical cells.

It has been shown that as well as having excitatory effects on spinal motoneurones corticospinal cells can have inhibitory effects. Lemon *et al.*, (1987) found that weak, single intracortical microstimuli were able to evoke post stimulus suppression of EMG activity in forelimb muscles in the conscious monkey. The latency of this suppression is consistent with there being an interneurone in the inhibitory pathway, and Jankowska *et al.*, (1976) has demonstrated that this interneurone is the Ia inhibitory interneurone.

The overall conclusions from these numerous studies employing various stimulating techniques appears to be that a single muscle can be activated by stimulation at many foci within the motor cortex. These foci represent "hot spots" of high density projections to target muscles. Also, stimulation within any one cortical site may activate multiple muscles (Phillips and Porter, 1977; Sato and Tanji, 1989).

Neurotransmitters of the motor cortex

The most likely excitatory neurotransmitters are aspartate and glutamate. In pigs, Potashner *et al.* (1988) showed that abalation of the sensorimotor cortex caused a specific depression of D-[^{3} H]-aspartate (a marker for L-glutamate and L-aspartate) uptake and release in the contralateral spinal cord. It is also known that glutamate

and aspartate are the primary neurotransmitters of projections from motor cortex to the striatum (Yamamoto and Davy, 1992). Also, corticothalamic and corticobulbar fibres have a high-affinity uptake system for d-aspartate and will transport it back to the cortical cell bodies (Rustioni and Cuénod, 1982; Jones 1984). Most of the glutamate and aspartate immunoreactive cell bodies are pyramidal cells in cortical layers III, V and VI. It has been shown recently that 65-75 % of identified corticospinal neurones exhibit this immunoreactivity (Giuffrida and Rustioni, 1989). There is some evidence that glycine may enhance NMDA-receptor mediated synaptic potentials in neocortical slices (Thomson *et al.*, 1989).

A major role for γ–Aminobutyric acid (GABA) as an inhibitory neurotransmitter in the motor cortex has been proposed. There is ample evidence to support this suggestion. This evidence includes the presence of specialised neurons that synthesize, contain, and release GABA (Iverson *et al.*, 1971; Otterson and Storm-Mathisen, 1984; Ribak, 1978). Also, intrinsic inhibitory postsynaptic potentials are blocked by specific GABA antagonists (Conners *et al.*, 1988; Dutar and Nicoll, 1988). Many of the cell types that are known to be GABAergic in the rat and non-human primate are present in the human, such as the chandelier cell (Marin-Padilla, 1987) and the basket cell (Marin-Padilla, 1969). There are two types of GABA receptors; $GABA_A$ (which have a post synaptic action) and $GABA_B$ (which have a presynaptic action). When electrophysiological studies are performed on the cortex inhibitory actions can be seen in the form of either early or late IPSPs. It is probable that the early IPSP are due to activation of $GABA_A$ receptors and the later IPSPs are due at least in part to activation of $GABA_B$ receptors (McCormick, 1989). There is data to suggest that GABA is a major inhibitory neurotransmitter in the human cerebral cortex and that GABAergic IPSPs play an important role in controlling the excitability and responsiveness of cortical neurons (McCormick, 1989).

Plasticity within the cortex

By defining the peripheral region that evokes a response in an identified neuron in the somatosensory cortex a topographic map can be obtained. These cortical maps are not static and can change in size and location throughout adult life. The cortical loci at which a given skin surface is represented can shift hundreds of

micrometers across the cortex (Clark *et al.*, 1988). These changes have been shown to occur in a number of cortical regions and can be provoked by a variety of situations in which the afferent input to the cortex is altered.

Somatosensory cortex

Merzenich and his colleagues (Merzenich, Kaas, Wall, Nelson, Sur, and Felleman, 1983) were the first group to demonstrate this capacity in adult mammals. They used microelectrode techniques to study the somatotopic organisation of the cutaneous representations of the hand in areas 3b and 1 of monkeys in which the median nerve had been transected and prevented from regenerating by ligation. The monkeys were investigated 2 to 9 months after the median nerve had been transected. They employed high resolution photographs of the cortical surface to enable the local vasculature to be used as an indicator of location. They discovered that the region of cortex that had previously been responsive to stimulation of skin areas innervated by the median nerve had become responsive to stimulation of skin surfaces innervated by the ulnar and radial nerves. These changes resulted in an increase in the size of the cortical area responsive to input from the ulnar and radial nerves. They also investigated the time course of these effects and discovered that the invasion on 'dennervated cortex' was a gradual phenomena and within about 3 weeks of nerve transection all or most of the former cortical territory of the median nerve is reoccupied. The conclusion from these experiments is that cortical somatosensory maps are dynamic and not static, and the cortical sites of representation of a given skin surface can change in time in adult monkeys. Other groups have reported similar findings in other mammalian species. Depending on the species and the mechanism of deafferentation the changes can be immediate and either reversible (Calford and Tweedle, 1988) or permanent (Pons, Garraghty, Ommaya, Kaas, Taub, and Mishkin, 1991). Often these changes are extensive with map expansions of many millimeters being seen (Pons *et al.*, 1991). This group did not use repeated recordings from intact animals as a control for ongoing changes in map stability, even though such changes have been shown to occur (Craggs and Rushton, 1976).

Motor cortex

It is now well established that similar changes in cortical representations can be seen in the primary motor cortex following changes in afferent input. Donoghue and colleagues (Donoghue, Suner, and Sanes, 1990) demonstrated, in adult rats, that the map of the motor cortex output was reorganised following a motor nerve transection. In their experiments the buccal and mandibular branches of the facial nerve were transected, and shifts in motor cortex output were tested by stimulating at a site in the vibrissa territory before and up to 10 hours after nerve transection. The stimuli employed evoked brisk vibrissa responses when applied in the low threshold vibrissa region without evoking any responses in the forelimb. Immediately following transection there was no evidence of EMG activity in forelimb muscles when these low threshold stimuli were given in the vibrissa region. However, within hours of the nerve transection stimulation in the vibrissa region elicited EMG responses in forelimb muscles that were similar to the responses obtained with stimulation in the pre-transection forelimb region. These mapping experiments indicated that the forelimb boundary had shifted approximately 1 mm into the former vibrissa region. Forelimb EMG could be evoked within the vibrissa region for up to 10 hours following the nerve transection. In an accompanying paper (Sanes *et al.* 1990) this group also demonstrated that similar changes in cortical organisation could be seen over a longer time-scale of 1 week to 4 months. Changes in cortical representation can also be brought about by repetitive stimulation of the motor cortex. Nudo and colleagues (Nudo, Jenkins, and Merzenich, 1990) used repetitive intracortical microstimulation in adult rats to demonstrate changes in the cortical representation. Cortical representations are also activity dependent. Humphrey *et al.* (1990) demonstrated that the forelimb motor representation in rodents cortex could be increased by passive movement of the limb.

Cues for reorganisation

The most likely trigger for motor cortex output reorganisation is an alteration in the somatic sensory inputs to motor cortex. It is known, for example, that somatic sensory afferent inputs influence neuronal discharge in rat motor cortex (Hummelsheim and Weisendanger, 1986; Sievert and Neafsey, 1986). It is also known that there is close correspondence between the afferent and efferent

organisation of the motor cortex (Rósen and Asanuma, 1972; Strick and Preston, 1982). It is possible that any change in the normal linkage between afferent and efferent systems could act as a trigger for reorganisation. Gellhorn and Hyde (1953) investigated this possibility by examining the effect of limb position on the motor cortex representation of monkeys. They concluded that the size of the muscle representation increased when the muscle was stretched, and suggested that this change was brought about by increased muscle spindle feedback. Also, Brons and Woody (1980) showed that repeated sensory stimulation of the glabella increases the excitability of motor cortex neurons that were synaptically coupled to neurons in the facial nucleus. Sanes *et al.* (1990b) examined the effect of changes in the configuration of forelimb upon the organisation of the rat motor cortex. Their results demonstrated that changes in sensory feedback could provoke major effects on the output map of the motor cortex and that these changes could take place over a very short period of time. The authors suggested that these changes included enhancement of the effectiveness of motor cortex linkages to the muscles as well as an expansion of the cortical area representing a set of muscles. It is difficult to differentiate between enhancement of effectiveness of linkage and an expansion of the map. Their evidence for an enhanced linkage appears to rest on the occasional finding of reduced threshold for motor responses to be evoked. I would like to suggest that this distinction is very difficult to make and I discuss it in more detail in a later experimental chapter (Chapter 7).

Mechanism of reorganisation in sensorimotor cortex

There appear to be two main mechanisms by which the observed changes in cortical organisation could be brought about and it may be that, depending on the time-scale of the observed changes, one or both of these mechanisms can play a part in the changes.

Some of the changes occur over very short periods of time and the very short time-scale of these changes suggests that the mechanism underlying them must rely on unmasking existing but inactive synaptic connections. This mechanism seems plausible when the magnitude of the change is considered. The cortical change is often, but not always within the extent of individual thalamocortical axon terminal fields, which can be 1 mm or more in width (Landry *et al.*, 1982, Garraghty *et al.*

1989). Therefore, expansion of representations could happen by increasing the effectiveness of synapses from the dense core to the sparse fringe, when the sparse fringe extends into the zone of deprived cortex. Also, lateral intrinsic connections within representations, which may be extensive, could also increase the effective zone of spread and changes relayed from one cortical layer to the next could lead to very extensive expansion (Kaas, 1991).

The favoured mechanism by which alterations in synaptic efficacy could be brought about is by alterations in the level of γ-amino-butyric acid (GABA) in the cortex. It is known that nerve section (Garraghty *et al.*, 1990), monocular deprivation (Hendry and Jones, 1986) and eye removal (Hendry and Jones, 1988) reduce the expression of GABA in the cortex. Dykes *et al.* (1984) showed that when the GABA antagonist bicuculline methiodide is iontophoretically administered to the somatosensory cortex of the cat the receptive field size of neurons increased. Matsumura *et al.* (1991) examined the effect of injection of a GABA agonist (muscimol) and GABA antagonist (bicuculline) into the precentral motor cortex while monkeys performed a reaction time task. They found that the performance of the reaction time task was unstable after injection of bicuculline. This instability was caused by increased EMG activity and by co-contraction of agonist and antagonist muscles. Reaction time was increased by injection of muscimol. Following injection of bicuculline the animals displayed muscle activity in response to a green light that did not require a response. In a later series of experiments this group (Matsumura *et al.* 1992) studied the activity of neurons in the motor cortex of monkeys while they pressed and released a lever in response to a visual cue. The effect of GABA, muscimol and bicuculline was examined and again it was apparent that reductions in GABA levels resulted in inappropriate neuronal activity. These findings suggest that GABAergic inhibition plays a role in regulating the population of task-related neurons, and the levels of the task-related activity. Also, GABAergic inhibition improves the directional specificity of cortical neurones. Jacobs and Donoghue (1991) performed a set of experiments to examine the effect of applying the GABA antagonist bicuculline to the forelimb region when stimulating in the vibrissa region of the motor cortex in adult rats. Initially stimulation in the vibrissa region resulted only in responses of the vibrissa. However, after administration of bicuculline at the

distant site it was possible to evoke both vibrissa movements and forelimb movements with the same stimuli in the vibrissa region. These results suggest that the application of the GABA antagonist bicuculline decreased inhibition and 'opened-up' existing but previously quiet synaptic connections within the cortex to forelimb muscles. These observations suggest that the receptive field of a cortical neuron is a dynamic phenomenon and can be modulated by factors that influence GABA-mediated inhibition.

Changes in cortical organisation have been reported which can occur over much longer periods (Sanes *et al.*, 1990a) and a different mechanism may be responsible for some of these changes. It is possible that the mechanism behind these changes relies upon new synaptic growth and connectivity. There is some evidence that new synaptic growth can occur in the central nervous system of adult animals (Raisman and Field, 1973; Tsukahara and Fujito, 1976). Recently Mano and colleagues (Mano, Kakamur, Tamura, *et al.*, 1995) presented some evidence of new synaptic growth in man following anastomosis of the intercostal nerve to the musculocutaneous nerve. This procedure was performed in subjects who had suffered brachial plexus avulsion in an attempt to produce some useful function in the dennervated biceps muscle. They were able to show, by using transcranial magnetic stimulation, that initially following the anastomosis the biceps muscle could be activated from the intercostal cortical area. At this time there was activity in the biceps muscle in time with respiration. Over a period of 1-2 years subjects were able to activate the biceps muscle independently of respiration. At this time responses could be evoked in the biceps muscle by stimulation of a more lateral scalp site, a site that approximated to the biceps representation on the non-operated side. This finding suggests that the biceps motor cortical region had gained control of some thoracic motor neurons. It is thought unlikely that motor regions that are as divergent as the biceps and intercostal could be anatomically connected (even by 'silent' synapses). However, there is some evidence to show that some corticospinal axons innervate both the cervical and thoracic cord (see Porter and Lemon (p147), 1993). These changes also took several years to develop and it therefore appears as if these findings provide some evidence that new synaptic connections can be formed.

Due to the time needed for the appearance of new connections it is extremely unlikely that this mechanism has a role to play in the rapid changes seen after deafferentation or learning, but may be responsible for some of the long term changes seen, for example, many years after an amputation. It is unlikely that it plays a role in the very rapid changes seen changes seen after deafferentation.

The basal ganglia

One region of the brain that has a great influence on the functioning of the cortical motor areas is the basal ganglia. This becomes very apparent in the disabling movement disorders seen when this region suffers damage.

The major part of the basal ganglia is made up of three nuclei: the caudate, putamen and globus pallidus. These nuclei are deep in the cerebrum, lateral to the thalamus and separated from it by the internal capsule. The globus pallidum is divided into two parts, lateral (or external, GP_e) and medial (or internal, GP_i). The lateral part is the larger, and although both parts look similar they make connections with different parts of the brain. The caudate and putamen are known as the striatum. There are two other nuclei in the basal ganglia. These are the substantia nigra and subthalamic nucleus and they lie in the midbrain. The subthalamic nucleus is found beneath the thalamus on the dorsomedial surface of the internal capsule. Ventral and caudal to this nucleus is the substantia nigra. The substantia nigra is also divided into two parts. The cells are more densely packed in the dorsal part of the nucleus. The dorsal region is known as the pars compacta and the ventral region as the pars reticulata.

The traditional view of the function of the basal ganglia was that it served to integrate influences from cortical regions, such as the 'association' and 'sensorimotor areas', as they passed through this region to targets in the thalamus. It was also thought that the zones within the thalamus that received basal ganglia output also received convergent input from the cerebellum and returned their own projections only to the primary motor cortex.

Recently ideas regarding the functioning of the basal ganglia have changed. It has been shown that basal ganglia and cerebellar efferents are directed to different targets within the ventrolateral thalamus (Asanuma, Thach, and Jones, 1983; Ilinsky, and Kultas-Ilinsky, 1987). Also, there is now considerable evidence that influences

from cortical association and sensorimotor areas remain separated through the circuits that link cortex and basal ganglia (DeLong and Georgopoulos, 1981). It appears as if there might be at least five basal ganglia-thalamocortical circuits which while running parallel remain segregated.

Basic circuit organisation

Each of the circuits mentioned above includes both a 'direct' and an 'indirect' pathway to the output nuclei. The 'direct ' pathway arises from inhibitory efferents from the striatum that contain both GABA and substance P (Albin, Young and Penney, 1989). Activation of this pathway disinhibits the thalamic targets leading to a cortical excitation. The 'indirect' pathway passes first to the external globus pallidus (GP_e) via striatal projection neurons that contain both GABA and enkephalin. From GP_e it projects to the subthalamic nucleus via a GABAergic pathway, and finally to the output nuclei via an excitatory, probably glutamatergic, projection. GP_e neurons have a high level of spontaneous discharge and exert a tonic inhibitory influence on the subthalamic nucleus. Activation of the projection from the striatum suppresses the activity of these neurones and thereby disinhibits the subthalamic nucleus, and increases its excitatory drive on the output nuclei. This in turn leads to increased inhibition of the target cells within the thalamus and a removal of facilitation of cortical cells. Therefore the two striatal projections appear to have opposing effects on the basal ganglia output nuclei.

Movement-related cells in the output nuclei can show either phasic increases or decreases in their normally high spontaneous rates of firing during specific motor acts. There is evidence that phasic decreases in the output discharge have a crucial role in motor control by disinhibiting the ventrolateral thalamus and thereby facilitating cortically initiated movements. Also phasic increases in the firing of output cells may have the opposite effect (Albin, Young and Penny, 1989; Scheel-Kruger, 1985; Klockgether *et al.*, 1985). Little is known about how inputs from the direct and indirect pathways interact to control basal ganglia output at the level of individual neurons within GP_i and SN_r. Overall, however, it is thought that the output from the basal ganglia may lead to a focusing of neural activity underlying cortically initiated movement (Alexander and Crutcher, 1990).

The motor circuit

The inputs to the motor circuit project principally onto the putamen. This region receives topographically arranged input from the primary motor cortex and other regions including the arcuate premotor area (APA) and the supplementary motor area (SMA) (Jones *et al.*, 1977; Kunzle, 1975, 1978; Selemon and Goldman-Rakic, 1985). The putamen also receives a topographical input from the somatosensory cortex (Jones *et al.*, 1977). These projections result in the leg being represented in the dorsolateral zone, an orofacial ventromedial zone, and the arm representation being seen between the other two regions (Kunzle, 1975; Crutcher and DeLong, 1984). It is possible that there may be separate SMA and motor cortex specific sub-channels within each of the somatotopically defined channels (Alexander and Crutcher, 1990).The topography is maintained between the putamen and its specific target portions of the globus pallidus and substantia nigra pars reticulata. These regions then send topographical projections to specific thalamic nuclei (Carpenter, Nakano, and Kim, 1976; Kuo and Carpenter, 1973; Kim *et al.*, 1976; DeVito and Anderson, 1982). The motor circuit is completed by the thalamocortical projection; VL_o and lateral Va_{mc} to the SMA (Strick, 1976), lateral Va_{pc} (also Vl_o) to the premotor cortex (Schell and Strick, 1984), and from Vl_o and CM to motor cortex (Strick, 1976; Kievit and Kupers, 1977; Wiesendanger and Wiesendanger, 1985). During stimulus triggered limb movements changes in neuronal discharge tend to occur in cortical regions of the motor circuit prior to changes in subcortical sites. This appears to suggest that much of the activity within these circuits is at least initiated at a cortical level (Alexander and Crutcher, 1990). However, there is temporal overlap of the bursts of movement related activity seen in cells at cortical and subcortical sites which suggests that much of the motor processing occurs in parallel.

Evidence has been presented that the motor circuit plays a part in the preparation for movement as well as having a role during the movement. It is well known that precentral motor fields contain neurons that show changes in firing rates when given an instruction to prepare for a directionally specified movement. Similar changes have been reported in the putamen (Alexander, 1987). The activity of neurons in the putamen and globus pallidus has been shown to be related to various aspects of movements, such as direction, amplitude, and load (Georgopoulos,

DeLong, and Crutcher, 1983; Crutcher and DeLong, 1984b; Liles, 1985). These studies suggest that the motor circuit of the basal ganglia might have a role in control of movement direction and in the scaling of movement amplitude. Neurons in these areas tend to be either related to preparation or movement, and this raises the possibility that there might be sub-channels responsible for different aspects of motor control within the somatotopic channels of the motor circuit (Alexander and Crutcher, 1990). Indeed, some recent work using retrograde transneural transport techniques lends support to this idea of sub channels within the motor circuit (Hoover and Strick, 1993).

It would appear then that the parallel circuits formed within the basal ganglia have a role in modulating operations of the frontal lobe and play an important role in the planning and performance of motor tasks.

Brain Stimulation

Stimulation of the exposed motor cortex has provided a great deal of information about the structure and function of this region of the brain. This technique has been of most use in the examination of cortical function in animal models. Over the last ten years or so the techniques of transcranial electrical and magnetic stimulation have been developed. Using these techniques of brain stimulation it is possible to study the function of the motor cortex in awake functioning human subjects. These stimulation methods have provided exciting new possibilities for the study of motor function both in health and disease.

Electrical stimulation of exposed cortex

Following an electrical stimulus to the exposed motor cortex a short latency muscle contraction is seen in contralateral distal musculature. In order to understand the mechanism by which this contraction comes about it is helpful to record from the spinal cord, and record the events seen following the cortical stimuli. The investigation of the nature of cortical stimulation became a great area of interest in the early 1950s (Patton and Amassian (1954), Lance and Manning (1954), Wall, Rémond and Dobson (1953)). Patton and Amassian (1954) performed some important experiments using surface bipolar stimulation of the motor cortex of cats and monkeys to record the descending volleys in the spinal cord. They found that a

single cortical stimulus could provoke a series of descending volleys separated by intervals of 1-2 ms. They proposed that the first of these volleys was due to direct activation of pyramidal neurones (at the initial segment or first node) and that the later volleys were due to indirect, transynaptic activation of pyramidal neurones. They labelled the first volley the D (direct) wave and the later volleys I (indirect) waves. Their evidence for suggesting these mechanisms consisted of the following observations: D waves survived asphyxia, injury or damage much better than I waves. Also, D waves could be activated selectively by stimulation of the underlying white matter. The size of the I waves was greatest when stimulating deep in the cortical grey matter. This finding that stimulation elicited differing responses depending upon the level of the stimulation was later confirmed by other groups (Landau, Bishop and Clare 1965, Asanuma and Sakata 1967, Jankowska, Padel and Tanaka, 1975a). Stimulation in the Betz cell layer resulted in both D and I waves in the pyramidal tract. Since stimulation in more superficial layers resulted in I waves without D waves, and repetitive stimulation produced marked facilitation of I waves but not D waves, it was concluded that intracortical microstimulation resulted in predominantly trans-synaptic activation of the pyramidal tract (Jankowska, Padel and Tanaka, 1975a). The orthodromic latency of a volley, recorded in the spinal cord, evoked from an intracortical stimulus was 0.5 to 1.5 ms longer than the antidromic latency from the same site in the cord to the cortex. This was taken as evidence of a synaptic delay in the activation of the pyramidal cell. Also, the temporal facilitation of I waves was typical of synaptic responses.

Using bipolar surface stimulation it is possible to activate pyramidal neurones with either anodal or cathodal stimulation. There are however a number of differences in the actions of the two forms of stimulation. Landgren, Phillips and Porter (1962a, b) investigated these differences using monopolar stimulation. They found that cells lying on the convexity of the gyrus were activated more readily (at lower threshold) than those in the wall of the sulcus. For the cells on the convexity the threshold for anodal stimulation was lower than that for cathodal stimulation. Also, low intensities of anodal stimulation were capable of producing pure D wave activation whereas cathodal stimulation and high intensity anodal stimulation both produced D and I wave activity. They suggested that anodal stimulation gave rise to

a vertical component of electric current flow which entered the dendrites of pyramidal neurones in superficial layers and left in deeper layers. Outward current flow at the initial segment or first node would cause depolarisation of the pyramidal neurone resulting in a D wave. When using anodal stimulation at higher intensities it was suggested that other cortical elements would be activated. This would result in transynaptic activation of the pyramidal cells and the generation of I waves. Cathodal stimulation would cause a reverse in current flow from that seen with anodal stimulation. It was suggested that this direction of current flow would be less advantageous for activating pyramidal neurone and so would lead to higher D wave thresholds.

Some years later Kernell and Wu (1967) extended these observations in the baboon. They found that: (1) single, strong, unipolar stimuli cause some pyramidal tract neurones to fire in both the D and I waves, and (2) there is a particular order of recruitment of the I waves. When using anodal shocks the first wave to be seen is a D wave. With increasing stimulus intensities either the third I wave (I_3) or I_2 are seen. With very high intensities of stimulation an I_1 might be seen. The interval between I waves is of the order of 1-2ms. This preferential order of wave generation was thought to be due to summation of EPSPs. The I_2 and later waves were able to benefit from summation with preceding EPSPs whereas I_1 would never be able to benefit from this mechanism. The pyramidal tract cell would also be refractory if it had fired during the D wave making it unresponsive to the I_1 bombardment. The periodicity of the waves has several possible mechanisms. Patton and Amassian (1954) attributed it to 'periodic bombardment of Betz cells through chains of neurones with fixed temporal characteristics'. However, this argument does not explain all the available data. For example, Kernell and Wu (1967) reported that there is remarkably little temporal dispersion of I waves recorded in the baboon (probably less than 0.5ms). This dispersion is less than might be expected from the suggested mechanism by Patton and Amassian. Phillips (1987) proposed another hypothesis. It is known that the impulse generating region of the pyramidal tract cell has pacemaker properties and can discharge repetitively in response to sustained inputs. The discharge frequency is related to the strength of the depolarising current, with the upper limit of frequency being determined by the refractory period of the

cell. Phillips suggested that the cell would discharge with a stereotyped pattern of firing when a large surface anodal stimulus was applied. A third possibility would be a combination of the two suggested mechanisms. There are many possible sources for the generation of the I waves. One possible source of I wave generation is by thalamo-cortical afferents. These afferents are known to terminate in layer III of the cortex and excite pyramidal cells. However, Amassian, Stewart, Quirk and Rosenthal (1987) lesioned the ventral thalamic nuclei which provide the major source of thalamo-cortical afferent fibres. Following this procedure they found that I waves were still able to be elicited. Another possible source of I waves are recurrent collaterals of large pyramidal cells. However, stimulation of the subcortical white matter only produces D wave activation and so it appears unlikely that this is the mechanism of I wave generation.

Two other possible major sources of I wave generation remain: intrinsic motor cortex circuits in deep cortical layers and extrinsic inputs from other cortical areas, such as premotor and somatosensory areas. At the moment it is unknown which of these mechanisms is the more important.

It is of interest to know whether the high instantaneous firing rates of pyramidal neurones seen in trains of D and I waves has any physiological importance. Pyramidal tract cells usually fire at frequencies of less than 100 Hz. However, the instantaneous firing frequency may be much higher. Lemon and Mantel (1989) reported inter-discharge intervals of 5 ms or less during phasic modulation of EMG activity in the monkey. Amassian *et al.* (1987) suggested that the cortical circuitry involved in the generation of multiple I waves, separated by precise intervals, did not evolve accidentally and may have a timing function. They proposed that the quantification of time by cortical interneuronal chains would permit a clock-like function in the motor cortex, and that such a function was necessary for precise regulation of ratios of angular velocities by synergists producing a specific trajectory.

Transcranial brain stimulation in humans

The techniques of stimulating the brain directly with electrical stimuli in anaesthetised animals and humans yielded much valuable information regarding the anatomy and physiology of the motor cortex. However, with the recently developed

techniques that allow the motor cortex to be stimulated in alert functioning human subjects significant advances in the understanding of movement control are now possible. The following sections describe these techniques of transcranial brain stimulation.

Transcranial electrical stimulation

Merton and Morton (1980) devised the first generally applicable method to stimulate the motor cortex of humans through the intact scalp. Large transient voltages of up to 750V were required. The stimulator needed to have a low output impedance in order to maintain the high voltage needed for stimulation. This stimulator was capable of delivering currents of above 1 A from a capacitative discharge with a time constant of 50 μs. This stimulator was the prototype of the commercially available Digitimer D180 stimulator. For this type of stimulation electrodes are attached over appropriate scalp locations with collodion and a conducting gel placed beneath the electrode cup. Merton and Morton's (1980) original stimulating montage consisted of the anode being placed over the hand area and the cathode being placed 4 cm anterior to the anode. With this type of stimulating montage they were able to obtain relatively focal activation of the contralateral hand. They later discovered that the stimulating voltage could be reduced if the cathode was placed over the vertex. With this montage current can spread to the opposite hemisphere and lead to activation of ipsilateral muscles. Rossini and colleagues (Rossini, DeStefano and Stanzione, 1985) in an attempt to obtain more localisation of the stimulus used a unipolar montage. The anode was placed over the hand area but the cathode consisted of a belt of ten or more equally spaced electrodes connected in a ring encircling the scalp. Some improvement in localisation was achieved and stimulus threshold was lowered. Cohen and Hallett (1988) improved the degree of localisation further by employing a closely spaced bipolar stimulating montage. The distance between the anode and cathode was only 2.5 cm. This stimulating pattern required high stimulus intensities but produced relatively good localisation.

Structures activated by transcranial electrical stimulation

Katayama, Tsubokawa, Maejina, Mirayama and Yamamoto (1988) have recorded a series of descending volleys in the spinal cord after stimulation of the

exposed motor cortex in man. They employed either a monopolar surface anode or a bipolar array to stimulate the cortex and a bipolar recording electrode placed in the epidural space to record the descending volleys. These volleys were similar to the D and I waves recorded in the monkey. Low intensities of stimulation resulted in a single volley that had a conduction velocity of 50-70 m/s. When higher stimulus intensities were used the initial volley was followed by further waves separated by intervals of 1.5-2 ms. As the later waves were susceptible to the effects of anaesthesia, it was suggested that these later waves were equivalent to the indirect (I) waves seen in the monkey. The conduction velocity of the I waves was the same as that of the D wave. When the opportunity has arisen to record from the exposed spinal cord in man (for example during spinal surgery) several groups have recorded similar waves when employing transcranial electrical stimulation (Boyd, Rothwell, Cowan, Webb, Morley, Asselman, and Marsden, 1986; Pelosi, Caruso and Balbi, 1988; Inghilleri, Berardelli, Cruccu, Priori and Manfredi, 1989; Berardelli, Inghilleri, Cruccu, and Manfredi, 1990; Burke, Hicks and Stephen, 1990).

It has been reported that with increasing intensities of stimulation it is possible to see a reduction in latency of the cord recorded potential. This reduction in latency occurs in clear steps and not in a gradual manner. Boyd *et al.* (1986), Katayama *et al.* (1988), and Inghilleri *et al.* (1989) found maximum reductions in latency of 0.3-0.5 ms. With a conduction velocity of 60 m/s this would mean that the site of stimulation had jumped to a site approximately 18-30 mm deeper into the white matter. Burke *et al.* (1990) found an even more striking reduction in latency in some of their patients with a two step reduction in latency of 0.8 and 1.7 ms being seen. This would equate with stimulation sites that would be approximately 5 and 10-11 cm deeper in the brain. They suggested that this would fit with stimulation in the internal capsule and cerebral peduncle respectively. Transcranial electrical stimulation appears to produce similar findings in the monkey as those seen in man. Edgley, Eyre, Lemon, and Miller, (1990) demonstrated shifts in latency in macaque monkeys, with latency and collision study data suggesting a site of stimulation as far caudal as the medulla.

In summary, it is possible to record D and I waves with transcranial electrical stimulation in man and the monkey. Using low intensity it appears as if there is

preferential D wave activity. Employing higher intensities of stimulation results in clear shifts in latency of the descending volley which are consistent with stimulation of the pyramidal tract a more caudal site. When higher stimulating intensities are used I wave activity is also generated.

Single motor unit studies

As well as using recordings from the spinal cord to monitor descending volleys evoked by cortical stimulation it is possible to examine the effects of these volleys on the motoneurone pool by using the technique of post stimulus time histograms (PSTH). This technique examines the firing probability of single motor units following cortical stimulation and gives information regarding the synaptic input to spinal motoneurones. Day and colleagues (Day, Dressler, Maertens de Noordhout, Marsden, Nakashima, Rothwell and Thompson, 1989) employed this technique to examine changes in firing probability of voluntarily activated single motor units in the first dorsal interosseous (FDI) after electrical stimulation over the motor cortex. They found that a cortical shock of threshold intensity produced a single sharp rise in firing probability starting 20-25 ms after the stimulus. With increasing stimulus intensity the size of this peak increased and then began to saturate. At higher intensities still the initial peak was followed by a second peak approximately 4-5 ms later. These results confirmed earlier findings (Calancie, Nordin, Wallin, and Hagbarth, 1987; Zidar, Trontelj, and Mihelin, 1987) that a single stimulus to the brain could elicit multiple peaks of increased firing probability in the PSTH of single motor units. It is suggested that these peaks are compatible with multiple EPSPs at the spinal motoneurone (Day *et al.* 1987a, b). The interval of 4-5 ms between the two peaks of the PSTH corresponds reasonably well with the interval between the D wave and the first recruited I wave (I_3) seen by Kernell and Wu (1967) in the monkey. On occasions an earlier peak was seen which had a latency that was consistent with the I_2 of Kernell and Wu (1967).

Effect of polarity of stimulation

Merton and Morton (1980) reported that anodal stimulation was effective at lower stimulus intensities than was cathodal. Boyd *et al.* (1986) and Burke *et al.* (1990) found that the threshold for evoking D waves was higher using cathodal stimulation than when using anodal stimulation. Other differences were noted when

comparing the two forms of stimulation. Day *et al.* (1989) reported that cathodal stimulation at threshold intensities was more likely to produce multiple peaks of increased firing than was anodal stimulation. They suggested that this finding was consistent with the known lower threshold for I wave production when cathodal stimuli are applied to the surface of the brain in a monkey (Hern, Landgren, Phillips and Porter, 1962).

The question arises as to what descending pathways are activated by transcranial electrical stimulation? When surface anodal stimulation is used to stimulate the cortex in awake human subjects a short latency response is seen in contralateral muscles. The latency of these responses is consistent with conduction in rapidly conducting corticospinal neurons (Rothwell *et al.*, 1987). It has been inferred from post stimulus time histogram studies that the rise times of the excitatory postsynaptic potentials generated in motoneurons are short, which suggests that the corticospinal neurons activated by anodal stimulation in man make monosynaptic connections with spinal motoneurons (Zidar, Trontelj and Mihelin, 1987; Day *et al.*, 1989). However, it is difficult to rule out some contribution from di-synaptic pathways.

Transcranial magnetic stimulation

During the period 1978-1982 Barker in Sheffield was developing a magnetic stimulator that was capable of activating peripheral nerves. At the end of this period he and his colleagues published a paper in which they described how they were able to stimulate human peripheral nerves (Polson, Barker, and Freeston, 1982). The technique of magnetic stimulation works by discharging stored electrical energy held in a capacitor through a coil of wire producing a magnetic field pulse. The magnetic field produced is very brief and typically reaches its peak value after approximately 0.1 ms and the current induced is proportional to the rate of change of the magnetic field. The induced current is induced in tissues beneath the coil which is then able to activate neuromuscular tissue in the same way as electrical stimulation.

In May 1985 a prototype magnetic stimulator was supplied by Barker's group to Merton and Morton at the National Hospital for Nervous Diseases, London. They attempted to stimulate the motor cortex of human subjects with this stimulator and were immediately successful, with the first records of motor responses to magnetic

brain stimulation being obtained (Barker, Jalinous and Freeston, 1985: Barker, Freeston, Jalinous, Merton, and Morton, 1985). This technique had one immediately apparent advantage over transcranial electrical stimulation in that it was pain free. Magnetic fields of the frequencies employed for this technique of brain stimulation pass through all body structures, including the skull, with little if any attenuation and hence can stimulate without producing painful sensations. This results in much lower electrical current flow at the scalp surface, and therefore less local muscle contraction and stimulation of small diameter nerve fibres in the scalp (two factors thought responsible for much of the pain associated with electrical stimulation).

For a circular coil the optimal placement for activating the intrinsic hand muscles is over the vertex. The direction of current flow in the coil determines which hemisphere is preferentially activated. Anti-clockwise current preferentially activates the left hemisphere and clockwise current activates the right hemisphere (Hess, Mills and Murray, 1987). The current flowing in the brain is in the opposite direction to that flowing in the coil. Unlike transcranial electrical stimulation, magnetic stimulation with a vertex centered coil will not produce any vertical component of current flow. This is perhaps a slightly unexpected finding but can be explained in the following way. Using mathematical modeling it has been shown that the magnetic vector potential (arising from the coil current) does have a vertical component. However, this magnetic vector potential induces a charge on the surface of the brain and the electric field from this charge appears to cancel the vertical component of the induced field at all depths. (Tofts, 1990).

The coil used for the majority of the experiments described here is a "figure of eight" double coil. The current flow is directed towards the handle, with the greatest field density being underneath the bar formed by the intersection of the two wings. The full technical details of the coil and stimulator are given in the General Methods section. This coil when placed over the hand area with the handle pointing posteriorly will induce a current in the brain that is in a similar direction to that produced by the round coil centered over the vertex i.e. flowing in a posterior to anterior direction parallel to the brain surface, with virtually no current flowing radially. This suggests that the magnetic stimulator excites horizontal neural elements. Mills *et al.* (1992) suggested that the neural elements stimulated are

aligned with their major axes at about 50^{0} to the saggital plane. This would correspond to horizontal fibres that were aligned approximately at right angles to the main axis of the motor strip. There is evidence of fibres in the motor cortex that are oriented in this direction. Jones and Wise (1977) found that the axon branches and dendritic fields of type 1 cells are orientated in an anterior-posterior direction i.e. at right angles to the long axis of the pre and post-central gyri in the monkey cortex. Marin-Padilla (1970) have demonstrated horizontal fibres orientated in the antero-posterior direction in layers IV and V in the human. It is possible that magnetic stimuli activate such a horizontal system and that the activation is maximal when the induced current is parallel to the main orientation of the fibres. It is also possible that the magnetic stimulus might activate pyramidal cells in the bank of the central sulcus either at the cell bodies or initial segments.

Defining the point at which stimulation takes place within the brain presents a very complex problem. The brain is not a homogenous conductor, with different tissues and irregularities in structure (i.e. sulci) being present. However, some attempts at modeling have been made and a number of important points have emerged regarding the action of magnetic stimuli. One of the most important of these was made by Amassian *et al.* (1992) who demonstrated the importance of bends in nerves when considering the ease with which they could be stimulated. Fibres running parallel to a uniform electric field are poorly stimulated. However, if they bend and deviate away from the plane of the field then it becomes possible for outward current to flow out of the nerve fibre thereby producing excitation. This finding has important consequences when considering cortical anatomy and the presumed site of activation of the axons of pyramidal cells. Fibres that follow paths which bend through induced electric fields are very likely to be preferentially activated. Maccabee (1993) demonstrated that the spatial derivative of the outward current is an important factor in determining the effectiveness of stimulation of a nerve.

When considering the point at which magnetic stimulation activates the nervous system the question of depth of stimulation has to be addressed. Rudiak and Marg (1994) used mathematical modeling to examine this question and reported that from their models threshold magnetic stimuli probably would act at the level of the

grey and white matter interface or even deeper within the white matter. Using electrophysiological techniques Baker and colleagues (Baker, Olivier and Lemon, 1994) showed that, at least in the monkey, the likely site of activation was at or very close to the pyramidal cell body.

Differences between transcranial electrical and magnetic stimulation

Using the conventional stimulating parameters there is one clear difference in the results obtained with transcranial electrical and magnetic stimulation. The latency of EMG responses in active hand and arm muscles is 1-2 ms shorter when using electrical stimulation.

Why this difference exists is still debated. Day *et al.* (1989) studied this difference by the use of single motor unit recordings. On the basis of their findings they suggested that electrical stimulation was capable of stimulating the axons of pyramidal cells directly whereas magnetic stimulation activated the pyramidal neurones transynaptically. Thus because there was a synaptic delay involved with the magnetic stimulation, electrically evoked responses were seen at a shorter latency. The implication was that electrical stimulation produced D waves and magnetic stimulation did not. Day *et al.* (1989) reasoned that this difference was brought about in the following way. Although most of the pyramidal cells innervating hand muscles are located in the rostral bank of the central sulcus, it is likely that some cells will also be found on the surface of the precentral gyrus. A threshold anodal stimulus will act directly on the vertically orientated pyramidal cells on the surface of the cortex to produce a D wave (Phillips and Porter, 1977). Magnetic stimulation which will produce current flow in grey matter parallel to the surface of the brain will be ineffective in activating vertically orientated pyramidal neurones but will preferentially activate horizontally orientated elements. These horizontal elements may activate pyramidal neurones transynaptically. Support for these ideas came from Amassian and colleagues (Amassian, Eberle, Maccabee, and Cracco, 1992) who used a peripheral nerve immersed in a brain shaped volume conductor to model the site of activation of both electrical and magnetic stimuli. They proposed that the orientation of the coil is the important factor in determining the site of activation produced by magnetic stimuli. Using a coil position that produced a posterior to anterior current flow through the hand area of cortex their results unequivocally

implied that the increased latency seen with magnetic stimulation was due to indirect activation of the axons of pyramidal cells. The differences in latency between electrical and magnetic stimulation are not apparent when examining responses in the lower limbs (Iles and Cummings, 1992), and their model implied that a round coil arranged tangential to, but not centered at, the vertex was capable of activating foot motoneurones.

An alternative explanation for the observed differences in latency of responses evoked in hand muscles with electrical and magnetic stimulation has been proposed by several other groups. Edgley *et al.* (1990) proposed that a vertex tangentially orientated magnetic coil and a transcranial electrical stimulus both activate pyramidal neurones directly. With suprathreshold stimuli electrical stimulation can activate pyramidal neurones at a deeper site (as deep as the medullary pyramid). Edgley *et al.* (1990) by the study of corticospinal volleys found that magnetic coil stimulation elicited I waves in the corticospinal tract at a higher threshold than D. However, in their later study examining responses in single corticospinal axons they showed that many corticospinal tract neurons actually had lower thresholds for an I response (Edgley *et al.* 1992). These apparently conflicting results can be explained by the much greater 'jitter' seen in the I waves in the axons. Due to this jitter these responses only sum weakly to produce a surface volley. Recently, Rothwell *et al.* (1994a) demonstrated that threshold transcranial electrical stimuli activated the pyramidal neurones at or near the level of the cortex. Thus, although the latency of the electrically evoked corticospinal tract response could be shortened by increasing the stimulus intensity (Burke *et al.*, 1990) this cannot account for the observed difference in latency when electrical and magnetic stimuli are applied at threshold intensities. Burke *et al.* (1993) performed some further studies examining the corticospinal volleys evoked by both electrical and magnetic stimuli in human subjects during orthopaedic operations. They found that both forms of stimulation evoked D and I waves, with the D waves having the lowest threshold. They reported that I waves were, however, more easily evoked with a magnetic stimulus than with an electrical one. In addition, the D wave evoked with a magnetic stimulus was smaller than that evoked by an electrical stimulus. These results might be taken as evidence to suggest that the observed difference in latency between the

two forms of stimulation seen when recording from hand muscles is not due to electrical stimulation producing a D wave and magnetic stimulation only producing I waves. However, as they point out themselves there are several complicating factors in these experiments. Firstly, many of the recordings were taken when the subjects were under the influence of volatile anaesthetics. These substances have a profound effect on the volleys (especially magnetically evoked) produced by brain stimulation. Secondly, many of the stimuli produced descending activity which was recordable in the thoracic cord. It is possible that this is produced by activation of pyramidal cells in the leg region of cortex. We know that the differences in latency are not seen when recording from the lower limb (see above) and so it may be that differences in the volley to hand muscles was being masked by volleys being produced by lower limb cortical cells. Another complicating factor is that by using different orientations of coil it is possible to get alterations in the balance between I and D waves. Werhahn *et al.* (1994) showed that a coil orientated in a medio-lateral direction over the motor cortex was better at producing D waves than was an anterior-posterior coil orientation. In summary, at the moment it is still unclear as to the exact site of activation of the two forms of stimulation.

An electrical stimulus to the exposed motor cortex can produce inhibitory as well as excitatory effects. The most effective stimuli are direct cathodal cortical shocks. These shocks are able to cause inhibition without first producing excitation (Krnjevic, Randic, and Staughan, 1966). Transcranial electrical stimuli are also able to produce inhibitory effects. Calancie *et al.* (1987) demonstrated that a transcranial electrical stimulus produced an excitatory response followed by a period of suppression of background EMG activity. Transcranial magnetic stimulation evokes a similar period of relative silence in the EMG when given during a tonic contraction. The basis of this period of suppression has been investigated by several groups. Using magnetic stimulation and H reflex recordings Fuhr, Agostino and Hallett (1991) concluded that the silent period depended initially on spinal mechanisms and subsequently on interruption of cortical drive. Inghilleri and colleagues (Inghilleri, Berardelli, Crucci, and Manfredi, 1993) investigated the silent period employing transcranial magnetic and electrical stimulation of the cortex, electrical stimulation of the cervico-medullary junction, and peripheral nerve

stimulation. They concluded that the silent period produced following cortical stimulation was due to several mechanisms. In the first 50 ms spinal factors such as recurrent inhibition and afterhyperpolarisation, are responsible for the suppression. The later period of the silent period, they believe, is due to inhibitory effects at the level of the cortex. The threshold for the inhibitory effect is lower than that to produce the excitatory muscle response (Calancie *et al.*, 1987; Davey *et al.*, 1992).

It has been shown that the volley produced by magnetic stimulation utilises the same population of corticospinal fibres as the volley produced by a transcranial electrical stimulus (Edgley *et al.*, 1992). As described in the section on transcranial electrical stimulation it is thought that this form of stimulus produces a monosynaptic excitation of target motoneurons (Palmer and Ashby, 1992).

Some evidence has been presented recently to suggest that later components of the evoked response to transcranial magnetic stimulation might be caused by a di-synaptic excitation conducted in a spinal propriospinal system (Gracies, Meunier and Deseilligny, 1994). It is suggested by these authors that this di-synaptically mediated excitation might make a significant contribution to the evoked EMG potential. However, this finding was for forearm muscles and there is no evidence for this mechanism contributing to the responses in hand muscles.

Magnetic stimulation as a tool for investigating change in cortical excitability.

Magnetic stimulation has gained widespread use for investigation of the functioning of the nervous system. By the use of some modifications in technique it is possible to obtain indications of alterations in the cortical representations of muscles. It is also possible to gain measures of the efficacy of inhibitory actions within the cortex.

Cortical mapping using transcranial magnetic stimulation

Magnetic stimulation has been used as a tool for producing maps of the cortical representation of muscles. Typically a grid of scalp sites is marked out on the scalp which covers the region from which responses in the target muscle can be evoked. The points in the grid are often separated by 1 cm in both the anterior/posterior and medial/lateral directions. Magnetic stimuli are then applied,

using a figure of eight shaped coil (which allows more focal stimulation than a conventional round coil) randomly to each scalp site and the evoked responses in the target muscle are recorded. The average size of the response following three to five stimuli at each site is calculated. This value is then used to produce a 'map' of the cortical representation of the muscle. Using separations of 1-2 cm and an eight shaped coil it is possible to differentiate the representation of proximal and distal arm muscles (Brasil-Neto *et al.*, 1992a; Wasserman *et al.*, 1992b).

Cohen *et al.* (1989) have shown that the results of this technique correlate well with those obtained with direct cortical electrical stimulation. Wasserman *et al.* (1992b) have shown that the cortical output maps obtained with this technique represent stimulation of the anterior bank of the central sulcus by projecting the results of magnetic stimulation mapping experiments onto the subject's brain magnetic resonance images. Also, it has been shown that this technique provides reliable and repeatable data (Mortifee *et al.*, 1994) and therefore it is concluded that this technique is useful for providing data on the cortical locus of muscle representations.

It is of interest to obtain a measure of the primary motor cortex area in order to compare the maps produced with magnetic stimuli to the underlying cortical anatomy. It is difficult to obtain a numerical value for this area of cortex but by measuring on a two dimensional picture (England and Wekely, 1991) I have approximated the area of the motor cortex to be approximately 5 cm^2. This value represents the area of cortex in one hemisphere that is visible from above and does not take into account any cortex that is in a fold (i.e. in the anterior bank of the central sulcus). From these measurements the motor strip is approximately 0.8cm wide and extends 6cm laterally from the midline. The posterior edge of this region is approximately 0.7cm anterior to the vertex.

Magnetic stimulation as a technique for studying change in cortical excitability

Temporary deafferentation of the forearm in humans leads to a rapid modulation of the output maps obtained from stimulation of the motor cortex. Brasil-Neto *et al.* (1992) employing the technique of transcranial magnetic stimulation investigated the effect of a period of transient forearm ischaemia on cortical excitability. Motor evoked potentials (MEPs) were recorded in the arm

muscles before, during and after a period of ischaemia. The MEPs from muscles proximal to the block gradually increased during the anaesthesia and then returned to pre anaesthesia levels within 20 minutes of the end of the ischaemic period. During the time when the MEPs increased in size MEPs recorded in the contralateral (control) arm did not change.

Similar changes are seen in subjects with 'permanent deafferentation'. Using transcranial magnetic stimulation Hall, Flament, Fraser and Lemon (1990) demonstrated that in two congenital amputees, following magnetic stimulation, responses on the amputated side could be elicited at lower threshold and from a larger cortical area than on the intact side. They also examined two traumatic amputees. In one of these who had suffered an early amputation they found similar results. However, in the second traumatic amputee who had only recently suffered the amputation there was no evidence of altered cortical output. Cohen and colleagues (1991) investigated a larger group of seven traumatic amputees and one subject with congenital absence of the hand. They found that magnetic stimulation evoked larger MEPs, recruited a larger percentage of the motoneuron pool, and elicited MEPs at lower stimulus intensities in muscles ipsilateral to the stump than in those on the intact side. They concluded that these results were compatible with cortical or spinal reorganisation of motor pathways targeting muscles proximal to the stump. Similar findings have also been reported in subjects with spinal cord injuries (Levy *et al.*, 1990: Topka *et al.*, 1991). Fuhr and colleagues (1992) investigated this phenomenon in subjects who had undergone amputation of the lower limb and found similar results. Using tests of spinal cord excitability (H-reflex testing) they demonstrated no increase in spinal excitability and proposed that the changes seen were due to alterations in cortical excitability.

The cortical representation of muscles also appears to be activity dependent. Pascual-Leone *et al.* (1995a) have demonstrated that the cortical representation is increased to muscles involved in a keyboard playing task after several days of 2 hour practice. This same group also demonstrated that the cortical motor output map was increased in Braille readers following a period of Braille proofreading (Pascual-Leone *et al.*, 1995b). In another study Pascual-Leone *et al.* (1993) reported that detection of a stimulus applied to the reading finger of a trained Braille reader was

blocked by a magnetic stimulus applied to a larger contralateral scalp area than that seen in control non-trained Braille readers. They suggested that these findings were consistent with expansion of the sensorimotor cortical representation of the reading finger.

Double-pulse magnetic stimulation

Recently several techniques employing transcranial magnetic stimulation have been developed which examine cortical inhibitory actions (Ferbert *et al.*, 1992; Kujirai *et al.*, 1993;). These techniques utilise double magnetic pulses to demonstrate inhibitory functions between regions of the cortex.

Corticocortical inhibition

For this technique two magnetic pulses are applied through the same coil placed over the hand area of one motor cortex. The first pulse is a sub threshold conditioning shock and the second pulse is a suprathreshold test shock (which produces a test response of approximately 1 mV in the contralateral first dorsal interrosseous muscle (FDI)). The effect of the first (conditioning) stimulus on the size of the response to the second (test) stimulus is investigated. When the conditioning stimulus precedes the test stimulus by short intervals (1-6 ms) an inhibition of the test response is seen. With longer interstimulus intervals (7-15 ms) some facilitation of the test response is observed. Kujirai *et al.* (1993) presented evidence that the inhibitory effect seen using this technique is cortical in nature. Firstly, they found that a magnetic conditioning stimulus over the hand area failed to produce any inhibition of responses evoked in minimally active hand muscles by small anodal stimuli. The following discussion may help explain this finding; It is probable that with stimuli that are just suprathreshold magnetic stimuli (with a coil current flowing in the anterior-posterior direction) produce more I waves than electrical stimuli (Day *et al.*, 1989). I waves probably reflect transsynaptic activation of pyramidal cells. Therefore it appears as if in most subjects magnetic stimulation produces more transynaptic activation of pyramidal cells than does electrical stimulation. With this difference in the manner of activation of the two forms of stimulation the responses to magnetic stimuli should be more affected by changes in the level of cortical excitability than the responses to anodal stimuli. Therefore, a

differential suppression of magnetic and electrical test stimuli is compatible with a cortical inhibitory mechanism. The second piece of evidence comes from H-reflex studies. A sub-threshold conditioning stimulus applied over the hand area or the vertex never produces a period of inhibition in forearm H- reflexes even though they produce clear suppression of responses evoked in the same muscle by magnetic stimulation. Some caution has to be used when using this argument to explain the findings above as some groups have slightly conflicting results (see earlier). Also, if magnetic and electrical stimuli do produce different ratios of D and I waves then the EMG responses evoked by the two forms of stimulation might have resulted from different combinations of inputs at the motoneurone pool and it may be that the responses are not matched appropriately.

Transcallosal inhibition

This technique again utilises paired magnetic stimuli. Using two figure of eight coils a stimulus is applied bilaterally to the hand areas. A conditioning stimulus is applied prior to a test stimulus over the contralateral hand area. Both these stimuli are suprathreshold and would, alone, evoke a response of approximately 1 mV in the contralateral FDI muscle. Using this technique Ferbert *et al.* (1992) were able to demonstrate an inhibitory action between the two hemispheres and suggested that it reflected activity in transcallosal pathways. This inhibition was seen when the interstimulus interval was between approximately 6-16 ms. Voluntary activity produced alterations in the level of inhibition and a role for this inhibition in the maintenance of unilateral movements was suggested by the authors.

It appears, therefore, that these techniques allows an assessment of some cortical inhibitory actions in conscious man. These inhibitory actions may be local (intracortical) or between distant regions of cortex (e.g. transcallosal).

The following chapters outline a series of experiments conducted using the techniques of paired pulse inhibition, transcranial mapping, and intensity curve recording, to investigate the functioning of intracortical inhibitory connections in normal motor control and organisation, and their role in the genesis of abnormal motor activity

Chapter 2

GENERAL METHODS

Subjects

A total number of 53 control subjects and 42 patients were investigated for the experiments described in this thesis. The patient group consisted of 4 traumatic amputees, 12 cortical myoclonics, 15 focal dystonics, and 11 patients with Parkinson's disease. Full subject and patient details are given in the following chapters. The patients studied in this thesis were recruited and clinically assessed by neurologists working in the Human Movement and Balance Unit. The following neurologists were responsible for the recruitment and clinical assessments: for the Parkinsonian patients - Dr Rivka Inzelberg; for the dystonic patients - Dr Geoff Sheean; for the myoclonic patients - Dr Peter Brown and for the traumatic amputees - Dr John Kew.

Recording techniques

Silver/silver chloride disc electrodes were used to record EMG responses evoked by magnetic stimulation. The active electrode was applied over the muscle belly and the reference electrode was placed over an adjacent relatively inactive site. For example, in the case of FDI, the active electrode was placed over the middle of the muscle belly and the reference electrode was placed over the first metacarpophalangeal joint. Under each electrode was placed a small amount of conducting gel and the skin surface abraded slightly to reduce impedance. Responses were amplified and the signals fed via a CED1401 laboratory interface (Cambridge Electronic Design, Cambridge, U.K.) to a PC for 'off-line' analysis. The CED1401 uses a 12-bit A-D converter. A sampling frequency of 5 kHz was employed for the recording of all responses. Two different gains were often employed for the recording of muscle responses, one low gain channel (typically 1 mV/V, allowing a maximum input signal of 5 mV) and one high gain channel (typically 100 μV/V). A low frequency filter of 53 Hz (-3dB) and a high frequency filter setting of 3 kHz (-3dB) were used.

Stimulating techniques

Throughout the experiments described in this thesis a high power Magstim 200 stimulator (The Magstim Company, Dyfed, U.K.) was used. This stimulator

produces a basically monophasic magnetic pulse that has a 100 μs active segment and a total duration of less than 1 ms. The pulse has a relatively small but long lasting tail. The area of the tail is equal to the area of active segment and so no net current flows in the brain. It is assumed that there is no stimulating effect from the tail as it has a amplitude of less than 10% of the peak. For the majority of experiments described the magnetic stimulator was connected to a "figure of eight" stimulating coil (wings of coil have an external diameter of 7 cm). With this arrangement the peak magnetic field is 2.2 Tesla and the peak electrical field is 660 V/m. The induced current is maximal under the bar formed by the overlap of the two circular windings and is proportional to the rate of change of the magnetic field. The magnetic field strength decreases with distance away from the coil and with the stimulator and coil used for these experiments the field strength is approximately 0.8T at a distance of 20 mm below the coil. As described in the Introduction (Chapter 1) the induced currents are parallel to the surface of the brain and do not penetrate very deeply. It is probable that stimulation takes place at the level of the cortex and not at any subcortical sites (Baker, Olivier and Lemon, 1994).

With this type of figure of eight coil it is possible to obtain relatively focal stimulation of underlying neural structures. In the experiments described the current flow is directed into the handle of the coil. All stimulus intensities quoted correspond to the value seen on the display of the magnetic stimulator.

For the experiments utilising double magnetic pulse stimulation a Magstim Bistim module (The Magstim Company, Dyfed, U.K.) was used. This unit allows two MagStim 200 stimulators to be linked together to provide double pulses through the same coil. Due to the switching characteristics of this unit the resultant pairs of stimuli are reduced in power when compared to stimuli given from a single stimulator. This results in the intensity being approximately 30% less than the that reported on the stimulator displays. The power output from a stimulator connected to the Bistim unit is compared to that seen with a single Magstim 200 unit in Fig. 2.1. These measurements were made with a search coil placed directly beneath the stimulating coil. The stimulating coil was discharged at increasing intensities and the induced current in the search coil was recorded and the data collected via the CED

1401 (sampling frequency 50kHz). Five stimuli stimuli were delivered at each intensity and the peak response was measured.

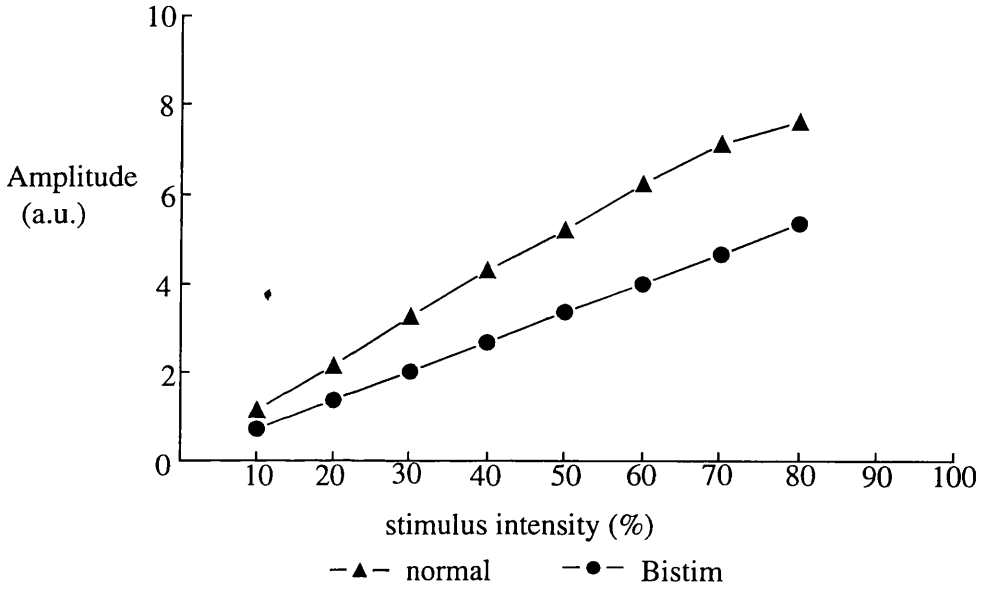


Fig. 2.1. The maximum amplitude of the induced current produced by either a standard high power Magstim 200 stimulator or when one these stimulators is connected to the same coil via the Bistim module. The amplitude is measured in arbitrary units and the stimulus intensity is expressed in terms of % output of the stimulator. It can be seen that across this range of stimulus intensities the output is approximately 30% less when the Bistim is connected.

The question arises as to whether there is any interaction of the two pulses when they are separated by very short intervals. Using a search coil as described above this possibility was investigated. As can be seen from Fig. 2.2A and B, at interstimulus intervals of greater than 1 ms there is no interaction of the two pulses produced from the BiStim. However, at an interstimulus interval of 1 ms there is an interaction of the pulses with the second pulse being slightly facilitated with respect to the first.

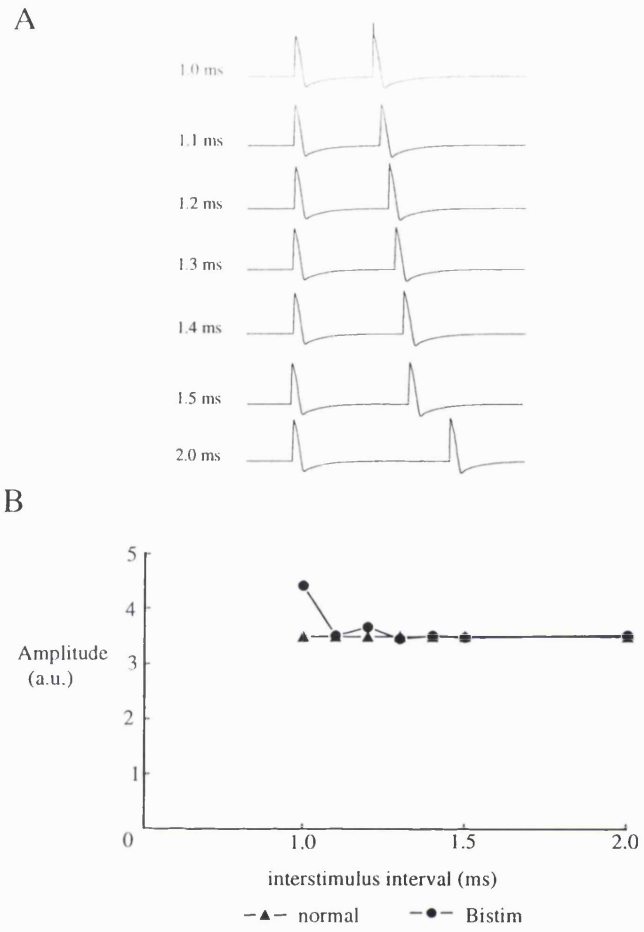


Fig. 2.2 (A) illustrates induced current pulses recorded when paired stimuli are delivered via the Bistim unit. Interstimulus intervals of 1.0-2.0 ms are shown. It can be seen that at the very shortest ISI (1.0 ms) there is some interaction of the stimuli, with the second stimulus being slightly facilitated with respect to the first. (B) illustrates this finding graphically, and again shows that the second stimulus is slightly facilitated when the ISI is 1 ms.

Coil orientation

For all the experiments described coil position was determined in a standard way. The coil was placed approximately over the hand area (7 cm lateral to the vertex) of the hemisphere contralateral to the test muscle with the coil in an anterior/posterior orientation (handle of coil towards pointing posteriorly). The coil was then moved until the point of lowest threshold for responses in the target muscle was found. The anterior point of the wing intersection was used as to standardise coil placement. Scalp markings were then made in order to enable accurate placement of the coil during testing (see Fig. 2.3).

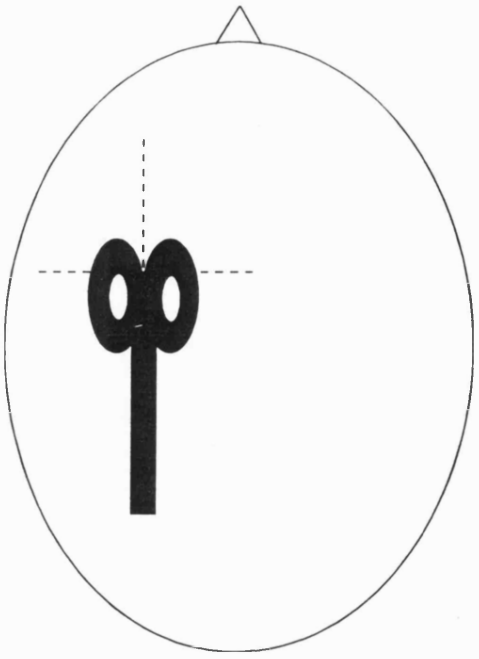


Fig. 2.3 illustrates coil alignment and placement with respect to scalp locations. The intersection of the dotted lines indicates a scalp location (for example a site at which a maximal responses are elicited from a target muscle).

Threshold determination

In many of the experiments accurate measurement of activation threshold was essential. It was necessary to determine threshold with both the target muscle relaxed and also when the subject maintained a small voluntary contraction. For determination of the relaxed threshold subjects were given audio-visual feedback from the target muscle to help them maintain relaxation. The coil was placed over the best site for stimulation and stimuli were the applied across a range of intensities. A rough approximation of threshold was determined by varying the stimulus intensity in steps of 5% of stimulator output from below threshold to above threshold. Once an approximation had been determined accurate measurement was obtained by varying the stimulus intensity in 1% steps. Threshold intensity was defined as being the intensity needed to evoke responses, of an amplitude of at least 50 μV, in 50% of ten successive trials. Any trials in which EMG activity was apparent were discarded and the subject was encouraged to relax fully. For the determination of threshold in active muscle, the subject was given audio-visual feedback to assist in the maintenance of the correct level of voluntary contraction.

The EMG from the target muscle was rectified, integrated and smoothed to give a level indicator of background contraction. Typically the level of background contraction was 5% of maximum voluntary contraction (MVC). A similar procedure to that described for relaxed muscle was then performed in order to determine the threshold. In the active condition it was necessary to superimpose or average responses to be sure of accurate threshold determination due to the ongoing EMG activity.

Repeated measurements of threshold in the same subject yielded values that were within 2 or 3 % (stimulator output) of earlier measurements. This was a consistent finding even if the measurements were taken days or even weeks apart.

Corticocortical inhibition

Cortical inhibition was evaluated using paired magnetic stimuli as described in detail by Kujirai *et al.* (1993). With the subject seated comfortably in a reclining chair paired magnetic stimuli were applied (via a Bistim module) through a figure of eight coil held over the motor cortex. One modification of the technique described by Kujirai *et al.* (1993) was employed in many of the experiments described in this thesis. We used a slightly lower intensity for the conditioning stimulus. This (conditioning) stimulus was set at an intensity 5% (of stimulator output) below the threshold for producing responses in minimally active (5% MVC) FDI. Kujirai *et al.*, (1993) employed a conditioning stimulus that was just subthreshold for a response in relaxed muscle. The second (test) stimulus was set at an intensity such that when it was given alone, it could evoke an EMG response, in the contralateral relaxed FDI muscle, of approximately 1mV peak to peak amplitude. On occasions corticocortical inhibition was investigated in both the active and relaxed conditions. Applying this criterion to both active and relaxed conditions meant that slightly lower intensities of test shock were used when subjects were active than when they were relaxed. The timing of the conditioning stimulus was altered in relation to that of the test shock. Interstimulus intervals (ISIs) of 1-15 ms were investigated. The experiments were conducted in blocks of 40 stimuli, with each block consisting of four different conditions; test alone and test + conditioning at three different ISIs. The order of presentation of these 4 conditions was generated pseudo randomly by the computer. The degree of randomness was set in the computer program and it was arranged so

that in any 8 trials there were 2 of each condition. This was done so that there were never long series of the same condition. The interval between successive pairs of stimuli was approximately 4-5 seconds. Measurements were made on individual responses and the area of the conditioned response, at each ISI, was expressed as a percentage of the area of the response to the test shock given alone. It was important for the subjects to be either completely relaxed or maintaining a minimal contraction of the target muscle. In order to help the subjects with this task they were given audiovisual feedback throughout the experiments. Any trials in which they failed to hold the correct activity level were rejected either on-line during the experiment or off-line during the analysis.

Transcallosal inhibition

This technique was developed by Ferbert and colleagues (Ferbert *et al.*, 1992). As stated in Chapter 1 paired magnetic stimuli are delivered using two figure of eight coils. Each of the coils was placed over the hand area of one hemisphere in an anterior/posterior orientation (handle pointing posteriorly). The intensity of both the conditioning and test stimuli was adjusted so that they evoked an MEP of approximately 1 mV in the relaxed contralateral FDI muscle. The testing routine was then similar to that used for the corticocortical inhibition measurements described above. For transcallosal inhibition ISIs of 6-16 ms were investigated. In these recordings the subject was encouraged to be totally relaxed and audiovisual feedback from both upper limbs was given to assist in this task. Again, measurements were made on individual responses and the area of the conditioned response, at each ISI, was expressed as a percentage of the area of the response to the test shock given alone.

Silent period

The silent period was elicited whilst subjects held a tonic voluntary contraction of approximately 5% MVC. Trials consisted of ten stimuli which were given approximately 5 seconds apart. Magnetic stimuli were applied using a figure of eight coil (described above) placed over the optimal scalp site for evoking responses in FDI and the responses were recorded using techniques described above. The duration of the silent period was assessed using at least two different stimulus

intensities (typically relaxed threshold and 20% (of threshold) above relaxed threshold). Audio-visual feedback was used to assist subjects in maintaining the correct level of activity throughout the trials. The onset of the silent period was taken as being the end of the MEP; the end of the silent period taken as the point where the first burst of EMG activity was seen following the period of EMG silence. In order to facilitate measurements the traces were often superimposed.

Statistics

The statistics used to analyse the results from the experiments conducted in this thesis are outlined in each experimental chapter.

Chapter 3

THE EFFECT OF VOLUNTARY CONTRACTION ON CORTICO-CORTICAL INHIBITION IN HUMAN MOTOR CORTEX

Introduction

Using the technique of paired pulse magnetic stimulation the excitability of intrinsic motor cortical circuitry has been studied (see Chapter 1). In previous experiments the excitability of the cortex was evaluated with the subject at rest. The aim of the present experiments was to examine whether the excitability of the cortex changes during performance of different voluntary movements. The changes observed are the first demonstration that the operation of corticocortical circuits changes during movement in conscious man.

Methods

A total of 14 normal subjects were studied: all gave informed consent and the procedures had approval of the local ethical committee. Six individuals participated in the main set of experiments. They consisted of one female and five males with ages ranging from 30-47 years (mean 35.3±6). EMGs were recorded from the first dorsal interosseous (FDI) muscle using techniques described in the General Methods chapter.

Corticocortical inhibition and thresholds were evaluated using the method described in the General Methods chapter.

In the main set of experiments corticocortical inhibition was investigated by in the relaxed condition (as described in General Methods) and also while subjects maintained a minimal voluntary contraction. In both cases the conditioning intensity was set to 5% below active threshold and the test intensity was adjusted to evoke an MEP of approximately 1mV in the contralateral FDI.

In eight subjects (3 female, 5 male, mean age 28±2.9 yrs) the effect of distant muscle activation on the level of ipsilateral corticocortical inhibition in the relaxed FDI was investigated. Subjects maintained a minimal (5%) tonic contraction of the ipsilateral biceps muscle, while keeping FDI relaxed and ipsilateral corticocortical inhibition was measured as described above. ISIs of 1, 2 and 3 ms were investigated. Visual feedback of the EMG level (using the high gain channel) was given to the subjects, via an oscilloscope, to assist in the maintenance of a consistent level of contraction. The test intensity was the same as in the main set of experiments (sufficient to evoke an EMG response of approximately 1 mV) and the conditioning

intensity was set at an intensity 5% of stimulator output below the threshold for evoking responses in the tonically active FDI.

A number of control experiments were performed using a variety of conditioning and test shock intensities. As in the main set of experiments FDI was the muscle studied. In the first of these experiments six subjects (4 male, 2 female, mean age 26±3.7 yrs) were studied at rest, using similar methods to those already described. Three ISIs at which inhibition is seen were used (1, 2 and 3 ms) and the degree of suppression of the test response was investigated at each of these different ISIs with varying test stimulus intensities. In each subject, the test stimulus was adjusted so as to evoke small (amplitude range 0.3 -1 mV) and large (2 - 4 mV) test responses. The second set of experiments was performed on three subjects (3 males, mean age 37±10 yrs). In these experiments the difference in the suppression of the test response when subjects were active or relaxed was compared (a) in the standard way, in which the size of the test shock was adjusted to produce responses of equal size in the two conditions, and (b) when the size of the test shock was constant in the two conditions. In the latter case, the test responses were larger than the standard 1 mV peak-to-peak. Interstimulus intervals of 2, 3 and 4 ms were investigated. The conditioning intensity was set at 5% of stimulator output below the active threshold (as in the main set of experiments). In the third set of experiments two subjects were studied and the effect of varying the conditioning intensity while keeping the test intensity constant was investigated. This was done with the target muscle (FDI) both relaxed and active. For these experiments ISIs of 1, 2, 3, 4, 7 and 10 ms were investigated. The conditioning stimulus was set at an intensity ranging from active threshold to 30% of stimulator output below active threshold.

Statistics

For the main set of experiments and also the control experiments the results were analysed using repeated measures analysis of variance. Student's paired t-tests were used in the main set of experiments to compare the results obtained in the active and relaxed conditions at individual ISIs. Unless otherwise stated data are given as means ± S.D.

Results

Comparison of paired-pulse effects in relaxed versus active FDI

These experiments were performed on the main group of six subjects. The mean conditioning intensity was 38±8(mean±SD)% of the maximum stimulator output as delivered via the Bistim unit (see Methods). The intensity of the test shock averaged 79±19% in the relaxed condition, but was reduced to 52±11% when the subjects were active.

Fig. 3.1A is an example of the effect of voluntary contraction on paired-pulse effects in a single subject. Traces show EMG responses in FDI to the test stimulus given alone and when conditioned by a subthreshold shock given 2 ms earlier. When the subject was relaxed, the test shock given alone produced a response which was approximately 1.0 mV peak-to-peak in amplitude. This was suppressed to 0.2 mV when conditioned at an interstimulus interval of 2 ms. When the subject was active, the test shock was adjusted so that it evoked an EMG response which was about 1.4 mV peak-to-peak. However, the conditioning stimulus (which was the same intensity as when relaxed) now had little effect.

The time course in Fig. 3.1B illustrates the mean data for all 6 subjects. When relaxed, the conditioning stimulus suppressed test responses at intervals of 1-5 ms, and enhanced them at 10 and 15 ms. When active, the time course was "flattened", i.e. there was less suppression and less facilitation. For analysis of the time course the ISIs were divided into two groups 1-6 ms and 7-15 ms. This was done because of the limited number of subjects in this set of experiments. This analysis revealed a significant ($F(1,5)=21.35$, $p<0.05$) state(active/relaxed)*ISI interaction. Individual paired t-tests revealed that during activation, there was a significant reduction ($p<0.05$) in suppression at intervals of 1, 2 and 3 ms and reduced facilitation at 10 and 15 ms. In this study we did not investigate the precise onset of the inhibitory effect. Because of interaction between the two stimulators it is not possible to study ISIs of less than 1 ms . However, from previous work (Kujirai *et al.* 1993) it appears as if the effect is not evident when the conditioning stimulus is applied 1 ms later than the test stimulus (i.e. ISI = -1 ms).

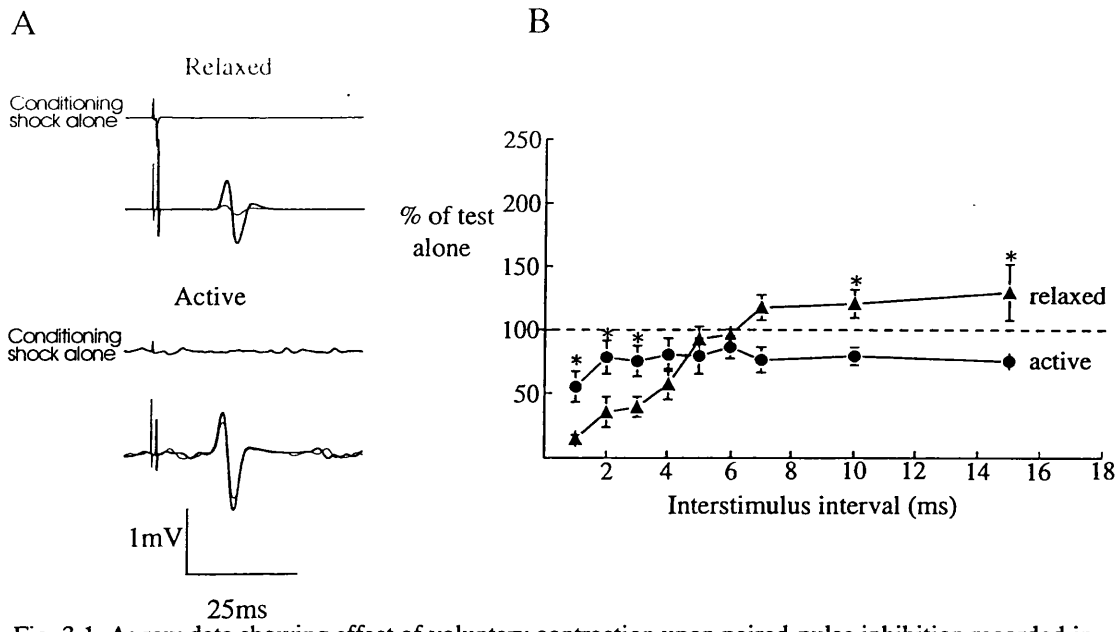


Fig. 3.1. A: raw data showing effect of voluntary contraction upon paired-pulse inhibition recorded in FDI at an ISI of 2 ms. Each trace represents the average response of ten trials. In each pair of traces, the upper record shows the absence of any EMG response to the conditioning shock given alone; the lower record shows the response to the test shock given alone (thick trace) superimposed on the response to the conditioning + test stimuli (thin trace). The conditioning intensity was the same in both the active and relaxed conditions and was set at 5% below the threshold for evoking responses in active FDI. The test intensity was set to give a test response in the active state that was similar in size to the one evoked when the target muscle was relaxed. When FDI is activated there is much less inhibition of the test response. B: time course of paired-pulse inhibition during both relaxation and a minimal tonic contraction of FDI. Each point represents the population mean (± standard error) from six subjects. The abscissa indicates the interstimulus intervals studied and the ordinate the area of the conditioned response as a percentage of the test response alone. It can be seen that over short ISIs (1-6 ms) there is less inhibition when the target muscle is active as compared to the relaxed state. Also, at long ISIs, there is less facilitation active as compared to relaxed (* $p<0.05$, paired t-test).

Effect of biceps contraction on paired pulse inhibitory effects in FDI

In eight subjects the effect of weak biceps (5% MVC) muscle contraction on paired-pulse inhibition in FDI was tested. With the aid of auditory and visual feedback, subjects maintained relaxation of FDI throughout the experiment. Intervals of 1, 2 and 3 ms between conditioning and test stimuli were examined since they had shown the greatest changes during contraction of FDI itself. Fig.. 3.2 illustrates that inhibition in FDI was the same whether biceps was relaxed or tonically active when considering intervals 1-3 ms ($F(1,7)=2.12$, $p>0.05$).

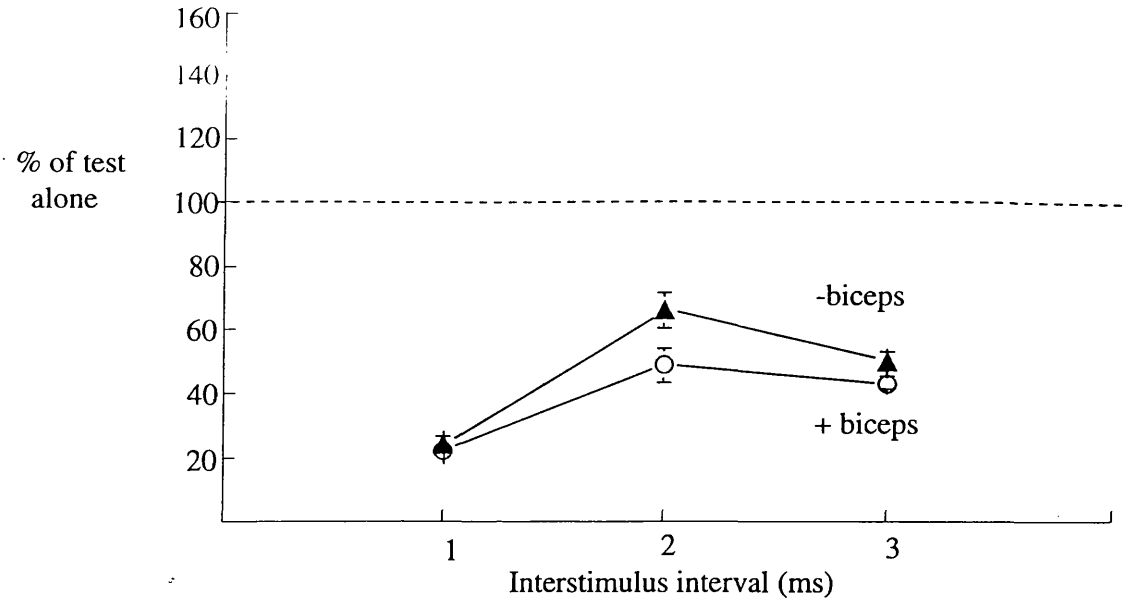


Fig.3.2. The effect of mild (5% MVC) voluntary contraction of the (ipsilateral) biceps muscle (+biceps) on paired pulse inhibition in relaxed FDI. Each point represents the population mean (±standard error) from 8 subjects. The abscissa indicates the interstimulus intervals studied and the ordinate represents the area of the conditioned response, expressed as a percentage of the response to the test shock given alone.

Effect of test shock intensity on paired-pulse inhibition

In the experiments above, the intensity of the test shock was adjusted to produce control EMG responses of approximately the same size when subjects were active or relaxed. The following experiments were performed to examine what effect varying the test response size had on the percentage inhibition produced by a constant conditioning shock.

Relaxed muscle

For relaxed muscle, the size of test response had no effect on the inhibition. The six (younger) subjects who participated in this series of trials had slightly lower mean thresholds (47±6% relaxed and 35±6% active, through the Bistim) than the initial group who were compared active and relaxed. In each subject, the conditioning intensity was kept constant (mean 30±6% stimulator output through the Bistim), whilst ISIs of 1-3 ms were investigated. In separate blocks of trials, 2 intensities of test shock were used in order to evoke EMG responses which lay within 2 broad amplitude ranges, 0.3-1.0 mV or 2.0 to 4.0 mV (mean test intensities of 53±6% and 60±10% (via the Bistim) respectively). Fig. 3.3A and B shows that

the conditioning stimulus produced the same percentage suppression for both sizes of test response ($F(1,7)=0.45$; $p>0.5$).

Active muscle

Again, the size of the test response did not alter the paired-pulse inhibition. In the initial experiments, intensity of the test stimulus was reduced when subjects were active so that it evoked an EMG response within the standard range 1-2 mV (peak-to-peak) amplitude. In three subjects the amount of inhibition using a test stimulus of equal intensity to that employed in the relaxed state was investigated. With the target muscle relaxed the test response had an amplitude of 1.2±0.53 mV (mean±SD) for the three subjects. In the active state with the usual (lower) intensity of test stimulus the test response had an amplitude of 0.97±0.25 mV, and with the higher test intensity (the same as that used when relaxed) an amplitude of 4.0±0.66 mV. In the relaxed muscle, responses were suppressed to an average of 28±1% of the test response alone. When the muscle was active, using the lower test intensity, responses were 83±24% of the test alone. With activity and using the higher test intensity responses were 100±21% of the test response. The percentage inhibition was not significantly different at any of the ISIs studied (2, 3 and 4 ms) in the two active conditions ($p>0.05$). An example from one of the subjects is shown in Fig. 3.3C.

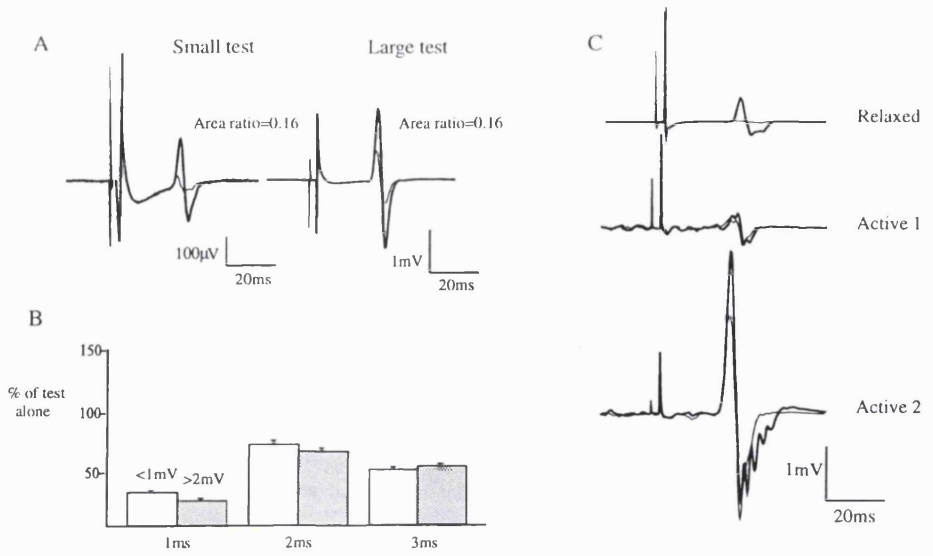


Fig. 3.3. Effect of changing the size of the test shock on the amount of paired-pulse inhibition. A and B refer to data collected when subjects were at rest. A shows raw data from a single subject. The superimposed EMG responses from FDI illustrate the size of the response to a test shock given alone (thick trace) and the response when conditioned by a conditioning shock given 3 ms earlier. In both cases the conditioning intensity is the same and was set at 5% below the threshold for evoking responses in active FDI. Two different test intensities were employed which resulted in test responses of 300 μV (left trace) and 3.4 mV (right trace; note calibration) being evoked. There was no difference in the percentage inhibition with the two sizes of test response. (On these averaged records it appears as if there is more inhibition in trials with the smaller test response. However, this is not the case and can be explained in the following way: the measurements quoted were made on single trials whereas the traces in this figure are averages of ten trials each. Because the conditioned response on the left has a complex morphology, the area of the average response may not be the same as the average of the areas of each single sweep.) The graph in B shows the population mean (±SE) data from six subjects. Three different ISI were investigated (1, 2 and 3 ms). The open bars show results when the peak to peak amplitude of the average test response was less than 1 mV. The filled bars show results when the test response was larger than 2 mV. The ordinate indicates the area of the conditioned response as a percentage of the test response alone. Across these three ISIs there was no significant difference in the degree of inhibition with the different test response sizes ($p>0.05$). C: raw data from one subject showing the effect of varying the test intensity in the active condition. The intensity of the conditioning shock was constant throughout. The top pair of traces shows that in relaxed muscle, the test response (thick line) is almost completely suppressed (thin line) when preceded by a conditioning stimulus given 3 ms earlier. In the middle trace (Active 1) the target muscle was active and the test intensity reduced so as to evoke an EMG response equal in size to that evoked in relaxed muscle (top trace). In this condition there was minimal inhibition of the test response. In the bottom trace (Active 2) the test intensity was the same as that used when the target muscle was relaxed. This resulted in a test response of much greater amplitude than in the relaxed state. With this size of test response, again, there was only minimal inhibition.

Effect of conditioning shock intensity

Fig. 3.4 shows the effect, in three subjects, of varying the intensity of the conditioning shock using a conditioning-test interval of 3 ms. When subjects were relaxed there was good suppression of the test response if the conditioning intensity was between active threshold and approximately 10% of stimulator output below active threshold. However, inhibition was still evident at 20% below active threshold. When the subjects were active, conditioning stimuli produced only minimal suppression of the test response. This was maximal, as in the relaxed state, when the conditioning intensity was set at approximately 10% below active threshold. Similar effects were observed at different conditioning-test intervals.

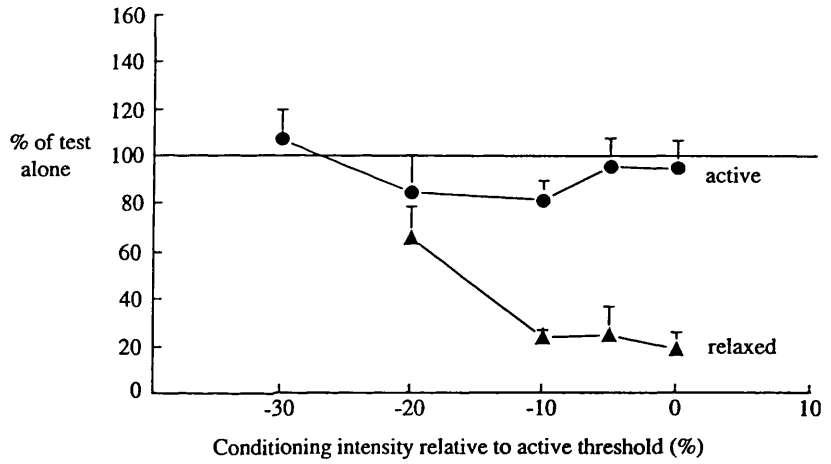


Fig. 3.4. The effect of varying conditioning intensity on paired-pulse inhibition in both relaxed and active FDI at an ISI of 3 ms. Each point represents the population mean value (±SE) of inhibition for three subjects. The abscissa indicates the conditioning intensity used, expressed in percent of stimulator output below or above active threshold. Active threshold was defined as 0%. The ordinate indicates the size of the conditioned response, expressed as a percentage of the test response alone. It can be seen that for relaxed FDI inhibition is maximal over a range from active threshold to approximately 10% below active threshold. In the active state inhibition is minimal across the whole range, but reaches its maximal level at approximately 10% of stimulator output below active threshold.

The time-course of paired-pulse suppression using different intensities of conditioning shock can be seen in Fig. 3.5A and B representing data obtained in two subjects. Fig. 3.5A shows the effect in relaxed FDI, while Fig. 3.5B shows the effect

in tonically active FDI. In both cases, maximum suppression is seen with conditioning stimuli which were 5-10% of stimulator output below active threshold.

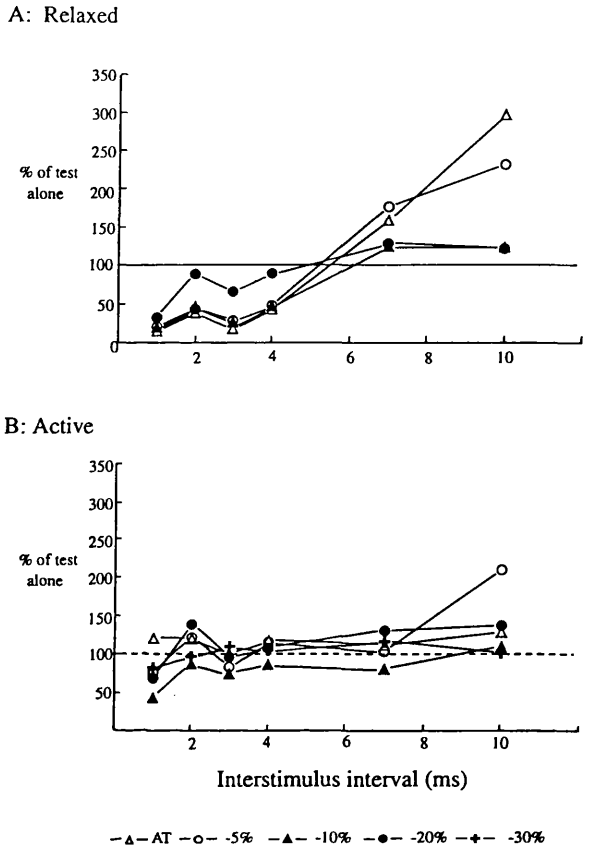


Fig. 3.5. The effect of varying conditioning shock intensity on paired-pulse inhibition across a range of ISIs, in relaxed FDI (A) and active FDI (B). Each point represents the mean data from 2 subjects. In the relaxed state the conditioning intensity was varied from active threshold (AT) to 20% (of stimulator output) below active threshold (-20%), and in the active state the conditioning intensity was varied from active threshold to 30% (of stimulator output) below active threshold. The abscissa indicates the interstimulus intervals and the ordinate the size of the conditioned response, expressed as a percentage of test response alone.

Discussion

These experiments have demonstrated that suppression, at short intervals, of a magnetically evoked test response by a subthreshold conditioning stimulus (corticocortical inhibition), is reduced during the maintenance of a minimal tonic contraction. It has been argued previously that this inhibition is cortical in nature (see Chapter 1).

Effect of changing the intensity of the test or conditioning shock

When the target muscle was active, lower intensities were used for the test stimulus in comparison with the relaxed state. This was done in order to match the size of the evoked EMG responses. It was possible that this difference in stimulating intensity might have been the reason for the observed difference in levels of inhibition between the two states. However, this is unlikely since the amount of inhibition was fairly consistent across a wide range of response amplitudes, at least in relaxed muscle. In addition, when the same test intensity was used in both the active and relaxed conditions there was no significant increase in the amount of inhibition during voluntary contraction.

In most experiments, the intensity of the conditioning shock was kept constant. However, this does not guarantee that the same population of cortical neurones was activated in the relaxed and active states. The threshold for transcranial stimulation of cortex is decreased during voluntary activity (Mazzocchio *et al.*, 1994) so that a constant conditioning stimulus may have activated a larger population of neurones than at rest. If so, this may have affected the amount by which the test response was suppressed. This seems unlikely to account for the findings as changing the intensity of the conditioning stimulus demonstrated that at all intensities the amount of suppression was less when active than when relaxed.

Effect of proximal muscle contraction

Although large changes were observed in the amount of cortical suppression during contraction of the target muscle (FDI), contraction of a remote muscle (biceps) had no significant effect. This indicates the changes were specific for the muscle studied and not simply the result of changing the task required of the subject. Indeed, since there was a slight increase in the amount of suppression during biceps contraction at all intervals tested, it may even be that contraction of remote muscles might facilitate cortical inhibition onto the target muscle. Whether this would be evident during contraction of muscles closer to the target requires further investigation.

Is the reduction in paired-pulse inhibition during voluntary activity due to changes in the excitability of cortical circuits ?

The results show that there is less paired-pulse inhibition when subjects are active compared with rest. Kujirai *et al.* (1993) argued that this inhibition is likely to be of cortical origin. The question remains as to whether a decrease in the amount of inhibition is due to changes in excitability at cortical or subcortical levels. In particular, could the conditioning stimulus cause spinal level facilitation when active but not when relaxed and hence partly compensate for an inhibited corticospinal volley ? It is not possible to study H-reflexes in the FDI muscle in order to investigate the effect of a sub threshold conditioning stimulus on spinal cord excitability but there are two reasons for rejecting this suggestion. First, it seems improbable that a stimulus intensity of 5% (of the stimulator output) below the threshold for evoking EMG responses in active muscle could produce any descending corticospinal activity. Second, if a minimal volley were responsible for producing spinal facilitation, then it should have been possible to increase the intensity of the conditioning stimulus when subjects were relaxed and obtain a similar decrease in paired-pulse inhibition. This was not the case: at rest, conditioning stimuli at an intensity as high as active motor threshold produced good inhibition which was no less than that seen with intensities of 15% (of stimulator output) lower. It would therefore seem that the reduced paired-pulse inhibition observed during voluntary activity is due to changes in excitability of cortical circuits. It is impossible to be certain whether voluntary contraction produces a decrease in excitability of inhibitory circuits or a concurrent increase in excitability of facilitatory circuits. However, there is one observation that favours the former possibility. Paired-pulse testing at rest revealed facilitation at longer ISIs (10 and 15 ms), which was absent during activity. Thus there was no evidence in the present data for increased facilitation and hence we suggest that the decreased paired-pulse inhibition during activity is likely to be caused by a reduction in excitability of cortical inhibitory circuits.

At these later ISIs the reduction in the level of facilitation of the test response during voluntary activity was significant at 10 and 15 ms. At the present time the mechanism of this later facilitation remains unclear and hence it would be prudent to keep the discussion directed primarily to the alterations in inhibition. Nevertheless, it

would not be surprising if voluntary contraction had effects on intracortical circuits other than those responsible for the initial suppression. However, further studies would be needed to tease these factors apart.

Comparison with previous work

It is known that transcranial stimulation over the motor cortex at intensities less than the threshold for evoking an EMG response can produce a short period of silence in the EMG of tonically active muscle (Calancie *et al.*, 1987; Davey *et al.*, 1992; Davey *et al.*, 1994). In a previous paper Kujirai *et al.* (1993) compared the inhibition revealed with paired-pulse testing at rest with low threshold inhibition of ongoing EMG and concluded that since the threshold for the effects was similar, they may share some common mechanisms. However, there were two points of difference between the techniques: the amount of suppression with paired-pulse testing at rest was shorter, but much deeper than the suppression of ongoing voluntary EMG (Davey *et al.* 1994). The present results with paired-pulse testing during voluntary activity help resolve the discrepancies. During contraction, paired-pulse testing results in less suppression, which lasts longer (suppression to approximately 80% seen at all ISIs tested from 1-15 ms) than when at rest. It seems likely that during tonic voluntary contraction, the effect of a single sub-motor threshold conditioning shock on both a subsequent larger test shock or on the ongoing EMG in a muscle is due to activity in similar cortical inhibitory circuits. Further support for this conclusion would necessitate comparison of the complete time-course of effects in both cases.

Chapter 4

CHANGES IN EXCITABILITY OF MOTOR CORTICAL CIRCUITRY IN PATIENTS WITH PARKINSON'S DISEASE

Introduction

The motor areas of the cerebral cortex are a primary target for the output of the basal ganglia, so that the deficits in movement control which occur in basal ganglia disease should ultimately be reflected in the activity of cortical cells. In monkeys, it has been reported that both electrolytic lesions of the substantia nigra (Gross *et.al.*, 1983), and treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Doudet *et al.*, 1990; Mandir and Watts, 1990) can produce substantial changes in the activity of neurones in the motor areas of the cortex. In the primary motor strip there is a decrease in the phasic discharge and a slower build-up of activity in cells with movement-related activity. In addition, there is a decrease in the proportion of cells which show reciprocal changes in their discharge in association with movements in opposite directions (Doudet *et al.*, 1990). Neurones in the supplementary motor area lose the "set-related" activity which occurs before the onset of movement in a known direction (Watts and Mandir, 1990). The aim of the present experiments was to identify changes in the excitability of motor areas of the human cerebral cortex in patients with Parkinson's disease.

Examination of motor cortical circuitry in man is necessarily indirect. Using transcranial electrical stimulation of the cortex, which activates corticospinal output axons in the white matter (Rothwell *et al.*, 1991), Dick *et al.* (1984) confirmed that the corticospinal projection was intact and readily accessible in patients with Parkinson's disease. More recently magnetic stimulation has been used (Kandler *et al.*, 1990). The EMG responses evoked by this technique are more sensitive to the level of cortical excitability than those evoked by electrical stimulation (Rothwell *et al.*, 1991), and may therefore reflect activity in cortico-cortical connections as well as in the output pathway itself. Cantello *et al.* (1991) reported that the threshold for magnetic stimulation was lower in patients with Parkinson's disease than in normals, but they did not distinguish whether this was caused by increased excitability of spinal motorneurones, which may have responded more readily to a given corticospinal discharge, or by an increase in the excitability of motor cortex neurones. Indeed, later studies have reported conflicting results, with some groups supporting a decrease in threshold (Cantello *et al.*, 1991; Maertens de Noordhout *et al.*, 1992) and larger responses (Eisen *et al.*, 1991), others an increase in threshold

but with larger responses at suprathreshold intensities (Davey *et al.*, 1991). The most recent study by Valls-Sole and colleagues (1994) has resolved some of these discrepancies by showing that when relaxed, the threshold for magnetic stimulation is the same in patients and normals, whilst the responses evoked at suprathreshold intensities are larger than normal. In contrast, when subjects are active, the responses in patients are likely to be smaller than normal. Valls-Sole *et al.* (1994) concluded that the effects are likely to be caused by changes in excitability of spinal cord (increased relative to normal at rest, decreased relative to normal when active) rather than cortical mechanisms. Measurements have also been made on the duration of the silent period which occurs in an actively contracting muscle after cortical stimulation (Valls-Sole *et al.*, 1994; Haug *et al.*, 1992). In normal subjects, the latter part of the silence is probably produced by inhibition of cortical activity, since spinal cord excitability as tested by H-reflexes recovers more rapidly than the EMG silence (Ziemann *et al.*, 1993; Fuhr, Agostini and Hallett, 1991). At high intensities of stimulation the silent period is slightly shorter than normal in Parkinson's disease which would be compatible with a reduction in the activity of cortical inhibitory circuits. However, the rebound in EMG activity which follows the silence is larger in Parkinson's disease than normal. This rebound may be the result of excitation of muscle afferents during the relaxation phase of contraction. If this reflex is enhanced in Parkinson's disease it may reduce the apparent duration of the silence.

As described in the Introduction to this thesis the technique of corticocortical inhibition may provide direct information on the excitability of cortico-cortical inhibitory connections within motor areas of cortex. The aim of the experiments in this chapter was to use this technique to examine possible changes in motor cortex excitability in patients with Parkinson's disease ON and OFF their normal therapy. In addition, measurements were made of both threshold and silent period duration for comparison with previous studies.

Methods

Patients

Eleven non-tremulous patients were selected from the out-patients clinic and their results compared with those from 10 neurologically healthy age-matched

control subjects. The patients were assessed by a neurologist (Dr Rivka Inzelberg). The age of the patients, the duration of their symptoms as well as their clinical ratings during OFF and ON states are shown in Table 1. The mean age of the patients was 65.3 ± 9.6 years whilst that of controls was 65.2 ± 9 years. All subjects were right handed and gave oral informed consent for participating in the study. The procedures were approved by the local Ethical Committee. The patients were investigated in both the OFF and ON conditions

Technique

Threshold, silent period and cortico-cortical inhibition measurements were made using the techniques outlined in the General Methods chapter (Chapter 2).

Transcallosal inhibition

This technique utilises paired magnetic stimuli to demonstrate inhibitory actions between the two motor cortices. This inhibition is thought to be mediated by transcallosal inhibitory pathways and the details of this technique are described in detail elsewhere (Ferbert *et al.*, 1992). In summary, magnetic stimuli were applied through two figure of eight coils (as described in General Methods section) placed over the hand area of the motor cortex bilaterally. The first stimulus was the conditioning and the second the test. Both conditioning and test stimuli were set to an intensity that evoked MEPs of approximately 1mV in the contralateral FDI muscle. Recordings were made with the target muscle (FDI) relaxed. Audiovisual feedback was given to the subjects to assist them in the maintenance of relaxation. Interstimulus intervals (conditioning-test) of 6 to 16 ms (in 2 ms steps) were investigated. Two blocks of 40 trials were recorded for each subject. Each block consisted of four different conditions; test alone and test + conditioning at three different ISIs. The order in which the conditions were presented was pseudo-randomly generated by computer. Individual responses were recorded and measured. The area of the conditioned responses were expressed as a percentage of the area of the test response alone. Relaxation of both FDI muscles was maintained throughout with the aid of audio-visual feedback.

Statistical analysis

The experiments were designed to consider two comparisons: between patients and normals and patients ON and OFF therapy. For ipsilateral corticocortical inhibition, the different interstimulus intervals (ISI) were divided into two groups: ISI=1-6 and ISI=7,10,15 ms (See General Methods chapter). The effect of GROUP (PD/control), ISI and the interaction between GROUP*ISI were analysed using Multivariate Analysis of Variance (MANOVA) for short and long ISIs separately. When the GROUP*ISI interaction was found to be statistically significant, the difference between the groups for each ISI was analysed individually using the Student's t-test. For transcallosal inhibition the effect of GROUP (PD OFF /control), ISI and the interaction between GROUP*ISI were analysed using MANOVA. The difference between OFF and ON states for different ISIs were analysed using MANOVA where the effects of ON-OFF, ISI and the interaction between ON-OFF *ISI were considered. Similar MANOVA models were used for analysing the GROUP differences for the silent period and the effect of stimulus INTENSITY as well as ON-OFF effects.

For the comparison of ON-OFF differences of Webster scores within PD patients, the paired Student's t-test was used. The correlation between several variables was evaluated using Spearman's correlation analysis.

Results

The clinical details of the patients are summarised in Table 4.1. Nine of the eleven patients were Hoehn & Yahr grade III, two were grade II. The Webster scores decreased significantly (paired t-test, $p<0.001$) when patients were ON compared with OFF therapy. Patients were able, with appropriate feedback, to maintain the correct level of background activity or relaxation during the recordings. If there were any trials in which there was inappropriate activity the trials were excluded from the analysis. In control subjects very low levels of background contraction have little or no effect on the degree of corticocortical inhibition seen (author's unpublished observations).

Patient	*sex*	*age*	**H&Y stage*	*disease duration(yrs)*	*Webster score OFF*	*Webster score ON*
1	*F*	*49*	*III*	*7*	*16*	*10*
2	*M*	*77*	*III*	*24*	*9*	*7*
3	*F*	*64*	*III*	*5*	*17*	*8*
4	*M*	*74*	*III*	*7*	*9*	*7*
5	*M*	*59*	*III*	*7*	*19*	*11*
6	*M*	*55*	*III*	*7*	*15*	*8*
7	*F*	*57*	*III*	*5*	*8*	*1*
8	*F*	*72*	*II*	*6*	*6*	*4*
9	*F*	*69*	*III*	*29*	*25*	*18*
10	*F*	*78*	*III*	*7*	*19*	*16*
11	*M*	*64*	*II*	*6*	*6*	*3*
			mean	*10.0*	*13.6*	*8.5*
			SD	*8.3*	*6.3*	*5.2*

Table 4.1 Clinical data from the 11 Parkinsonian patients studied.

** Hoehn and Yahr stage descriptions*

Stage I. *Unilateral involvement only, usually with minimal or no functional impairment.*

Stage II. *Bilateral or midline involvement, without impairment of balance.*

Stage III. *First sign of impaired righting reflexes. This is evident by unsteadiness as the patient turns or is demonstrated when he is pushed from standing equilibrium with the feet together and eyes closed. Functionally the patient is somewhat restricted in his activities but may have some work potential depending upon the type of employment. Patients are physically capable of leading independent lives, and their disability is mild to moderate.*

Stage IV. *Fully developed, severely disabling disease; the patient is still able to walk and stand unassisted but is markedly incapacitated.*

Webster Rating scale - see Appendix 1

Threshold values

The threshold stimulus intensity needed to evoke a minimal EMG response in the FDI muscle varied considerably (range: normals, relaxed FDI 41-83%, active 30-65%; patients, relaxed 47-75%, active 30-57%) from one individual to another. On average, it was the same in patients (ON or OFF therapy) as in normals, whether

the measurement was made with the muscle relaxed or active (see Fig. 4.1). As expected, the threshold was significantly higher in relaxed than in active muscle [MANOVA, INTENSITY effect, $p<0.001$].

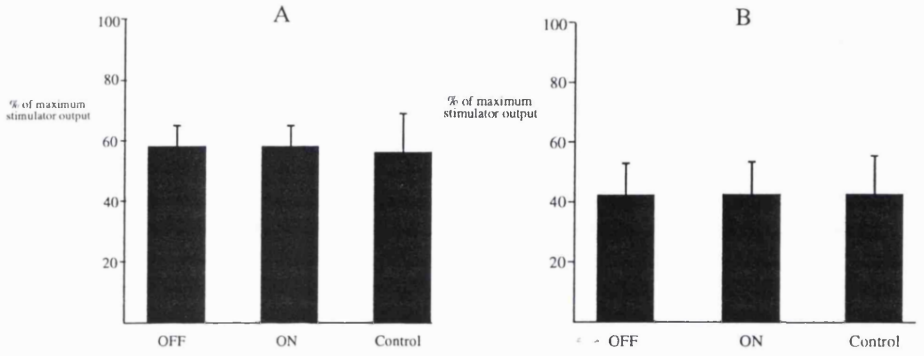


Fig.4.1 Bar chart showing thresholds for evoking EMG responses in (A) relaxed and (B) tonically active first dorsal interosseous (FDI) muscle to transcranial magnetic stimulation. Thresholds (y axis) are in % of stimulator output. The stimulation was performed with a Fig.. of eight coil placed over the hand area. Mean values ± standard deviations are given for the patients in both the OFF and ON states as well as controls. In neither the active nor relaxed states was the threshold significantly different in patients when compared to the controls ($p>0.05$).

Ipsilateral corticocortical inhibition

Ipsilateral corticocortical inhibition was investigated over interstimulus intervals from 1-15 ms. A comparison of the mean data from the patients studied OFF their therapy and normals is shown in Fig. 4.2A. Analysis of variance showed that the time course of the initial period of inhibition was different in the patients OFF from that in controls (ISI 1-6 ms; interaction term between group comparisons, $p<0.05$) with significantly less (t-test, $p<0.05$) inhibition at ISIs of 2, 4 and 5 ms. The behaviour of the patients was the same as that of normal subjects in the period of facilitation (ISI=7-15ms). When ON therapy, the patients' data at ISI 1-6 ms seemed to become closer to normal (Fig. 4.2B). There was no longer any significant difference between the amount of inhibition in patients ON v normal ($p>0.05$); but neither was there any significant difference between patients ON v OFF ($p>0.05$).

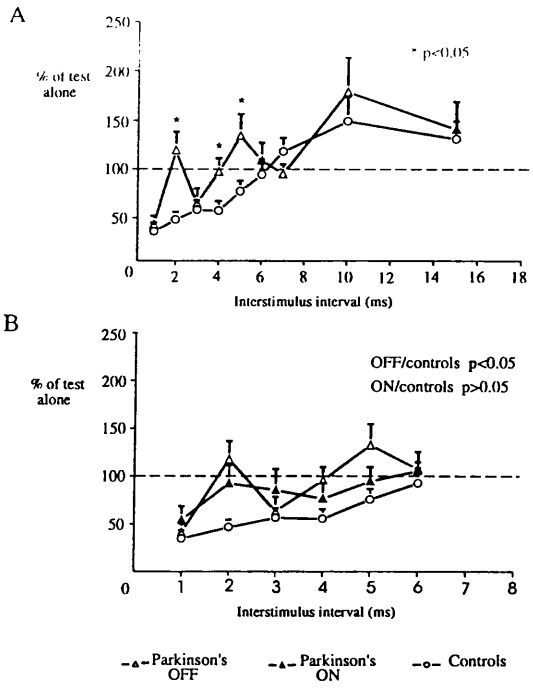


Fig. 4.2 Time course of ipsilateral cortico-cortical inhibition in the relaxed FDI muscle. The x-axis is the interstimulus interval (ISI) between the conditioning and the test shock ; the y-axis is the size of the conditioned response expressed as a percentage of the response size produced by the test shock given alone. The dotted line at 100% represents the size of the test response given alone. (A) data obtained at ISIs 1-15 ms from normal subjects and from patients in the OFF state . Intervals at which there was a significant difference (p<0.05, t-test) between the percentage inhibition in patients and normals are marked with an asterisk. (B) illustrates in detail data obtained for ISIs from 1-6 ms in patients OFF and ON therapy and in the normal control subjects. Each point represents the mean area (±SE) of the conditioned response in all subjects expressed as a percentage of the test alone response at that ISI. Across ISIs from 1-6 ms there was significantly less inhibition in the patients in the OFF state compared to normal (manova, GROUP effect, p<0.05). When ON the difference was not significant (p>0.05).

Transcallosal Inhibition

Transcallosal inhibition between the hemispheres was examined in the OFF condition in four patients. The time course of inhibition was the same as that in normals (Fig. 4.3) (MANOVA, GROUP effect, p>0.5). When treated as a separate small group, the ipsilateral cortico-cortical inhibition (ISI 1-6 ms) in these four patients was significantly less than that in controls (MANOVA, GROUP effect, p<0.05).

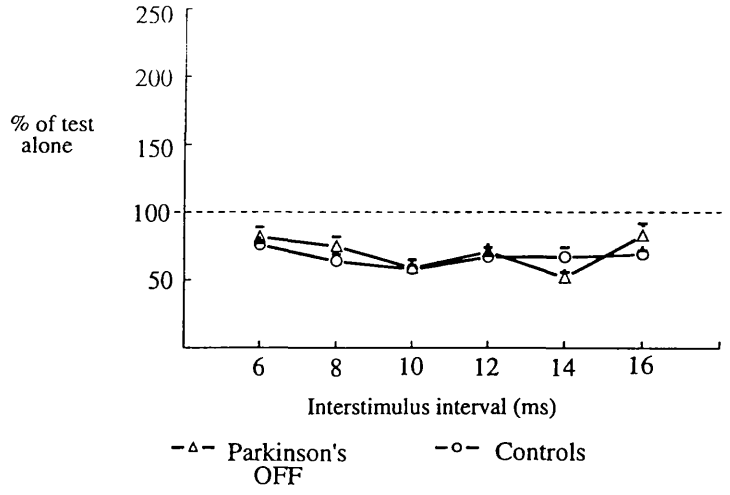


Fig. 4.3 Comparison of the time course of transcallosal inhibition in normal subjects and in 4 patients with Parkinson's disease studied OFF therapy. The x-axis plots the interstimulus interval (conditioning shock before test shock) and the y-axis plots the size of the conditioned response expressed as a percentage of the size of the response to a test given alone. Magnetic stimuli were given over both hand areas with figure of eight coils. There is no significant difference in the time course between the patients and the normals. Points represent means ±SE.

The silent period

The silent period was elicited at two intensities of stimulation: relaxed threshold and 20% of the stimulator intensity output above that value. An example of the silent period in one patient ON and OFF therapy is shown in Fig. 4.4A. The mean duration of the silence is shown for all subjects in Fig. 4.4B and C. The duration of the silent period increased with increasing stimulus intensity by similar amounts in all 3 groups of subjects (MANOVA, intensity effect, $p<0.001$; intensity-group interaction, $p>0.05$). The duration tended to be shorter in patients OFF therapy than in normals and longer than normal in patients when they were ON therapy, although neither effect was significant. Paired comparisons in individual patients showed that there was a significant ($p<0.05$) increase in duration (25 ms) ON v OFF when threshold intensities were used. The effect was not significant when stimulating at the higher intensities ($p=0.06$). There was no correlation between any of the individual clinical scores (rigidity, tremor, bradykinesia) or the global Webster score and silent period duration in the patients ON and OFF therapy.

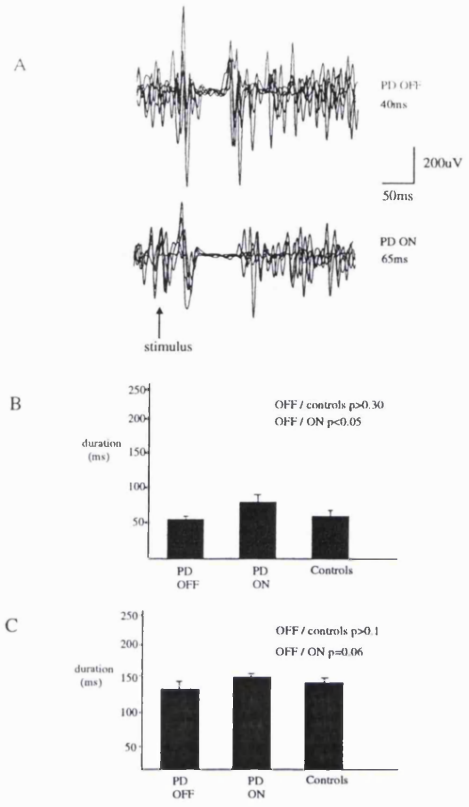


Fig. 4.4 (A) Superimposed raw data traces (5-trials) showing EMG silent periods in the contracting (5% MVC) FDI of a patient with Parkinson's disease. Cortical stimulation using a Fig.. of eight coil was given at an intensity equal to relaxed threshold. In the OFF state the silent period duration is shorter than in the ON state. (B) shows the mean data (±SEs) in the patients OFF and ON therapy as well as in the normal controls when the stimulus intensity was equal to relaxed threshold, and (C) when the stimulus intensity was equal to 20% of the stimulator output above relaxed threshold. With both stimulus intensities the silent period in the patients appears slightly shorter than in normals when OFF and slightly longer than in normals when ON. However, these changes are not significant. There is a significant ($p<0.05$, paired t-test) lengthening of the silent period when ON as compared to OFF when stimulating at threshold intensities.

Discussion

The present results show that there is a reduction in the amount of corticocortical inhibition, as tested using transcranial magnetic stimulation, in the cortical motor areas of patients with Parkinson's disease studied after overnight withdrawal of their normal medication. However, there was no significant difference between patients and control subjects in either (i) the threshold for eliciting EMG responses (whether active or relaxed), or (ii) in the duration of the EMG silent period

seen after cortical stimulation. In patients, L-Dopa improved the amount of inhibition, and lengthened the duration of the silent period.

For reasons already outlined in Chapter 1 it seems possible that the suppression of the test response seen when using paired magnetic stimulation arises in the cortex. Thus the reduction in the amount of suppression seen in patients with Parkinson's disease reflects changes in cortical excitability.

It is possible that many different cortical circuits, both excitatory and inhibitory, are tested using the paired pulse technique. Reduced suppression of the test response at short interstimulus intervals is consistent with either a decreased inhibition or an excess of excitation. However, since the net effect at short intervals is inhibition, it has been suggested previously (Kujirai *et al.*, 1993) that an important component may be due to activity in intracortical GABAergic inhibitory connections. If so, then the results in Parkinson's disease would be compatible with a decrease in excitability of these GABAergic pathways.

The details of the results deserve discussion. First, why is reduced suppression not evident at all the interstimulus intervals studied between 1 and 5 ms ? It is only possible to speculate on the reasons for this behaviour, and, given the limited number of subjects that we studied, it is probably best to be circumspect. However, it is possible that the differences represent varying interaction between excitatory and inhibitory circuits at the different interstimulus intervals. The second point concerns the normal transcallosal inhibition that we described in the patients. This result has two important implications. First, it suggests that the mechanism of transcallosal and ipsilateral inhibition are different, and second, that the changes in Parkinson's disease are specific to the latter. Precisely which neural pathways are involved is, at the present time, unknown.

How does deranged basal ganglia output in Parkinson's disease affect the excitability of the intrinsic connections within the motor areas of cortex ? One clue comes from the changes in motor cortical cell discharges seen in monkeys after administration of MPTP. In intact animals, approximately half of the cells which change their firing during flexion/extension movements of the wrist have a reciprocal pattern of activity for the two directions of movement. After MPTP treatment, this directional selectivity is reduced. Only 18% of cells have a reciprocal firing pattern. As a result, many neurones whose activity might have been expected

to decrease during movement in one direction continue to discharge (Doudet *et al.*, 1990). A similar effect is seen after local injection of the GABA antagonist bicuculline into the motor cortex. There is a decrease in the selectivity of neuronal discharge, so that neurones which normally discharge during active movement of a joint in only one direction begin to discharge during movements made in either direction (Matsumura, Sawaguchi, and Kubota, 1991). Again, there is a net increase in the cortical activity accompanying movement in either direction. It seems possible, therefore, that the changes in cell firing after MPTP could be due, in part, to abnormal basal ganglia input to cortical inhibitory circuits. If so, then the results from neural recordings in monkeys provide a direct link with the present data. Basal ganglia dysfunction in Parkinson's disease may decrease the excitability of cortical inhibitory circuits and result in reduced ipsilateral corticocortical suppression as tested by magnetic stimulation.

The results from experiments with local injection of bicuculline (Matsumura, Sawaguchi, and Kubota, 1991) suggest that one role of inhibitory connections within the motor areas of cortex is to 'focus' activity onto appropriate groups of corticospinal neurones. The present data suggest that basal ganglia output may be one important factor which regulates this 'shaping' process. Such a role would be compatible with other studies which have shown that basal ganglia output is unlikely to be the source of the initial command to move, but is more likely involved in preparing cortical motor areas for a forthcoming movement. In essence one role of the basal ganglia may be to pre-set excitability in cortical circuitry so that the movement is executed as efficiently as possible.

In the patients studied in these experiments, decreased corticocortical suppression may be related to two common features of Parkinsonian pathophysiology: enhanced long-latency stretch reflexes, and ON-dose dyskinesias. Both may result from activity in inappropriate populations of cortical cells to somatosensory and motor inputs. In effect, dyskinesias might result from an overflow of activity within motor cortex after restoration of motor command signals from other sources. Long-latency stretch reflexes, if they involve activity in a transcortical pathway (Marsden, Rothwell and Day, 1983), may be increased because of a similar increase in the population of cells responding to the sensory input. In contrast slowness of movement in Parkinson's disease is unlikely to result

solely from these changes in motor cortical organisation. Perhaps it relates to a seperate "energising" function of the basal ganglia in other cortical motor areas.

L-Dopa treatment improved the suppression produced by paired magnetic stimuli. The most likely explanation for this is that improved striatal function normalised basal ganglia output. However, the possibility that there was an additional direct effect on dopaminergic input to superficial cortical layers cannot be dismissed.

Comparison with previous work

Data on the threshold for cortical stimulation in patients with Parkinson's disease is rather confused, with some groups claiming a decrease (Cantello *et al.*, 1991; Maertens de Noordhout *et al.*, 1992) in threshold and larger responses (Eisen *et al.*, 1991), others an increase in threshold but with larger responses at suprathreshold intensities (Davey *et al.*, 1991) and others no change (Valls-Sole *et al.*, 1994). The present results support the latter view.

A probable reason for the lack of consistency in the results from different groups is that there is a very wide range of threshold values in the normal population. With such an intrinsically variable measurement, it is difficult to prove that definite changes occur unless the differences are very large (as, for example, in patients treated with anti-epileptic drugs (Hufnagel *et al.*, 1990)), or if very large numbers of subjects are studied. So far, relatively small numbers of patients have been studied, so that the question for Parkinson's disease must remain open. The results from these experiments suggest that if threshold changes do occur, then they are likely to be quite minor.

A lack of change in threshold does not necessarily conflict with the present observations on the reduction in corticocortical suppression. The interaction between the two depends critically on the tonic level of inhibition in subjects at rest. It may well be that inhibitory activity is low at rest and only changes during or in preparation for movement. If so, then excitatory thresholds would be unaffected by changes in the excitability of inhibitory circuitry. Indeed, inhibition of GABAergic transmission in monkey motor cortex produces little change in the background activity of cells when the monkey is not performing any task, even though the

discharge during activity is increased considerably (Matsumura, Sawaguchi and Kubota, 1991).

The present results showing that the duration of the cortically evoked silent period is increased by L-Dopa therapy are similar to those reported by others (Inghilleri *et al.*, 1993). Many factors contribute to this silence. There is good evidence, from the use of H-reflex testing, that the latter part of the silent period is the result of a lack of corticospinal input to the spinal cord rather than a decrease in excitability of spinal motoneurones (Zieman *et al.*, 1993; Fuhr, Agostini and Hallett, 1991). In view of this, it may be that the changes which occur are related to the reduced suppression detected in the paired stimulus experiments. However, the stimulus intensities used to elicit the silent period are far larger than those used to test ipsilateral corticocortical inhibition, so that the question must remain unresolved at the present time. Cantello *et al.* (1991) originally reported that the silent period was shorter in patients with Parkinson's disease than in age-matched normal controls. This was not the case in the present study. The probable reason for this is a methological one. In these experiments both a smaller range of stimulus intensities and a smaller coil were used than by Cantello *et al* (1991). In fact, they only found shorter silent period durations when high stimulus intensities were used. When they used lower intensities, probably similar to those employed in these experiments, there was no difference in duration. Similar results have recently been reported by Valls-Sole *et al* (1994).

In conclusion, the results of the experiments experiments described have shown that it is possible to demonstrate changes in motor cortex excitability in patients with Parkinson's disease. The principal effect was a reduction in the amount of ipsilateral corticocortical suppression as tested with paired magnetic stimulation. This may be due to a decrease in excitability of intrinsic inhibitory circuits of the cortex, caused by abnormal output from the basal ganglia. Such changes may result in decreased selectivity of cortical discharge during movement as reported previously in monkeys treated with the neurotoxin MPTP.

Chapter 5

CHANGES IN THE BALANCE BETWEEN MOTOR CORTICAL EXCITATION AND INHIBITION IN FOCAL, TASK SPECIFIC DYSTONIA

Introduction

Focal, task specific dystonia, of which Writer's cramp and Musicians dystonia are examples, is characterised by excessive muscular activation during fine manipulative tasks. In the case of writing this excessive activity usually is seen in muscles of the forearm and hand. On the rare occasions where pathology can be demonstrated it is usually confined to the putamen, caudate, thalamus and globus pallidus or their connecting pathways (Rothwell and Obeso, 1987). Writer's cramp is therefore believed to be a disease of the basal ganglia .

There have been several electrophysiological studies in patients with dystonia. Many of these have focused on the spinal or brainstem abnormalities. For example, a reduction in reciprocal inhibition between antagonist forearm muscles has been described in Writer's cramp (Panizza, Hallett and Nilsson, 1989; Nakashima *et al.*, 1989), while changes in the blink reflex recovery cycle have been seen in patients with blepharospasm and cranial dystonia (Berardelli *et al.*, 1985; Tolosa, Montserrat and Bayes, 1988; Nakashima *et al.*, 1990). More recently, however, several reports have addressed the possibility that pathophysiological changes may also occur within the cerebral cortex, particularly in the motor areas which are a primary projection target of the basal ganglia. The Bereitschaftspotential, which is thought to be generated in the supplementary and primary motor areas, is decreased (in its later phase) prior to self-initiated movements (Feve, Bathien and Rondot, 1994; Van Der Kamp *et al.*, 1995). During free-choice joystick movements PET studies have shown decreased activity in the SMA (Jahanshahi et al., 1995). Reilly *et al.* (1992) reported bilateral abnormalities of the N30 component of the somatosensory evoked potential. Finally, Thompson *et al.* (1994) reported that the duration of the silent period, following transcranial magnetic stimulation, was in the low normal range.

As described in the previous chapter, by using the technique of corticocortical inhibition it has been possible to show that the relative excitability of inhibitory circuits is reduced in Parkinson's disease, in which there is known pathology of the basal ganglia. In this chapter intracortical inhibition in the motor cortex of a group of patients with focal task specific dystonia (14 cases of Writer's cramp and one case of simple Musician's Dystonia) has been investigated. A

reduction in inhibition has been demonstrated as in the Parkinson's disease patients albeit with a different time-course. We suggest that the reduction in inhibition may be, at least partly, responsible for the symptoms of excessive and inappropriate muscle contraction which are characteristic of the condition.

Methods

Patients

With the approval of the local ethical committee we studied 15 patients with focal task specific dystonia (14 cases of Writer's cramp and one case of simple Musicians dystonia (47±13 years(mean ± SD)), and 8 neurologically normal age-matched controls (49±14 years) were studied. Patients and control subjects gave informed consent. All patients and controls were right handed. Four of the Writer's cramp patients were being treated with botulinum toxin. The patients were assessed clinically by a neurophysiologist (Dr Geoff Sheean) and were subdivided into two groups depending on their symptoms. If their symptoms were specific to writing and only involved muscles employed for writing they were termed as *simple* Writer's cramp. If their symptoms were specific to writing but also involved muscles not usually employed for writing (i.e. more generalised muscle involvement) then they were termed as *dystonic* Writer's cramp (Sheehy, Rothwell and Marsden, 1988). In 10 of the patients responses were also recorded from the left FDI following stimulation of the right motor cortex. Clinical details of the patients are given in Table 5.1.

Technique

Paired pulse inhibition and thresholds were investigated using the techniques described in the General Methods.

Patient	*age*	*sex*	*diagnosis*	*treatment*	*duration*
1	50	F	dystonic	BTX	9 yrs
2	67	M	dystonic	BTX	6 yrs
3	55	M	simple	BTX	10 yrs
4	74	M	tremulous	none	5 yrs
5	45	M	simple	BTX	28 yrs
6	38	M	musicians	none	2.5 yrs
7	34	F	dystonic	artane 60mg/d	9 yrs
8	54	M	dystonic	none	20 yrs
9	30	M	dystonic	none	1.5 yrs
10	44	F	simple	none	6 yrs
11	25	M	simple	none	3 yrs
12	56	M	dystonic	none	5 yrs
13	36	F	dystonic	none	2 yrs
14	48	M	simple	none	3 yrs
15	51	M	dystonic	clonazepam 0.5mg bd	11 yrs

Table 5.1 Clinical details of dystonic patients

Statistical analysis

In the analysis of the experiments we considered the following comparisons: (i) between patients left hemisphere and controls left hemisphere, (ii) patients right hemisphere and controls left hemisphere (iii) the left and right hemispheres of individual patients (iv) those patients who were on botulinum toxin and those on no medication, and (v) the level of inhibition in simple Writer's cramp patients and those patients with dystonic Writer's cramp. For the main set of experiments with paired pulses the effect of GROUP (patients/controls), ISI and the interaction between GROUP*ISI were analysed using repeated measures analysis of variance (MANOVA (SPSS Inc)). The comparison of the right and left hemisphere inhibition in the patients, the effect of botulinum toxin, and the comparison of inhibition in the two sub-groups of Writer's cramp patients (simple and dystonic) was performed using MANOVA. For comparison of threshold data in the patients and controls a t-

test was used. For comparison of right and left hemisphere thresholds in the patients the paired Student's t-test was used.

Results

Thresholds

In the relaxed condition, following left hemisphere stimulation, the threshold for the controls was 56±9% (mean ± SD) and 57±10% for the patients. The threshold following right hemisphere stimulation in the patients was 55±9%. When active, following left hemisphere stimulation, the threshold for the controls was 40±9% and for the patients 42±11%. Following right hemisphere stimulation the threshold in the patients was 40±8%. There was no significant difference in threshold in either the relaxed or active conditions when comparing left hemisphere stimulation in the patients and controls (relaxed state: t-test, $P>0.5$; active state: $P>0.5$). Also, no significant difference was seen in the thresholds for stimulation when comparing the right and left hemispheres in the patients (relaxed condition: paired t-test, $P>0.5$; active condition: $P>0.1$).

Paired pulse suppression

When the whole time-course (ISI 1-15 ms) was examined there was a significant difference between the patients left hemisphere and the control subjects left hemisphere (MANOVA, $p<0.01$). Also, there was a significant difference when comparing the patients right hemisphere with the control subjects left hemisphere (MANOVA $p<0.05$). In both of these cases there was no significant interaction between GROUP and ISI ($p>0.05$). The time-course of the inhibition can be seen in Fig. 5.1. It is known from previous studies that in normals the time-course can be divided into an "inhibitory phase" (ISIs 1-6 ms) and a "facilitatory phase" (ISIs 7-15 ms). On the basis of this division further analysis was performed on the data. When we averaged the data across ISIs 1-6 ms, the "inhibitory phase", there was significantly less inhibition both when comparing the patients left hemisphere with the control subjects left hemisphere (MANOVA, $p<0.001$) and also when comparing the patients right hemisphere with the control subjects left hemisphere (MANOVA, $p<0.01$).

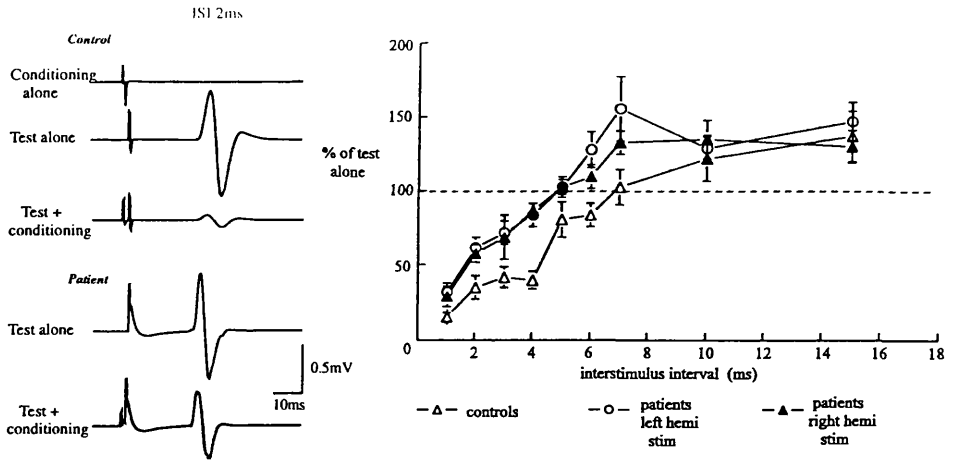


Fig. 5.1. Time course of paired pulse effects. (Left) illustrates raw data traces from a control subject (top set of three traces) and a representative patient with Writer's cramp (bottom set of two traces). This data was obtained following left hemisphere stimulation and responses recorded in the relaxed right FDI. In both the control subject and the patient the subthreshold conditioning stimulus evoked no response in the target muscle (see top trace), whilst the test stimulus evoked a clear EMG response of approximately 1 mV (peak to peak amplitude). In the control subject it can be seen that when the conditioning stimulus was given 2 ms prior to the test stimulus there was clear suppression of the response (bottom trace for the control subject). In the case of the patient there is much less suppression of the test response when conditioned at an ISI of 2 ms (see bottom trace). (Right) illustrates the data obtained across all ISIs in controls and patients (both left and right hemisphere stimulation). The y-axis is the area of the conditioned response expressed in terms of percentage area of the test response alone. The x-axis is the ISI between conditioning and test stimuli.

When the data was averaged across ISIs 7-15 ms, the "facilitatory phase", there was no significant difference comparing either the patients left hemisphere with the controls left hemisphere (MANOVA, $p>0.2$) or patients right hemisphere with the controls left hemisphere ($p>0.5$). Fig. 5.2 shows the average level of inhibition and facilitation across these blocks of ISIs. In the controls, conditioned responses were suppressed to an average of 50±15% (mean ± SD) of the test response alone across ISIs 1-6 ms following left hemisphere stimulation. In the patients responses were suppressed to only 80±17% following left hemisphere stimulation and 76±20% following right hemisphere stimulation. In the control subjects responses were facilitated to an average of 121±35% of the test response across ISIs 7-15 ms, while in the patients, responses were facilitated to 144±44%

following left hemisphere stimulation and 127±23% following right hemisphere stimulation. Botulinum toxin had no significant effect on the level of inhibition observed in the left hemisphere of patients (MANOVA, p>0.05). There was no significant difference in the level of inhibition when comparing the simple and dystonic Writer's cramp patients (MANOVA, p>0.5).

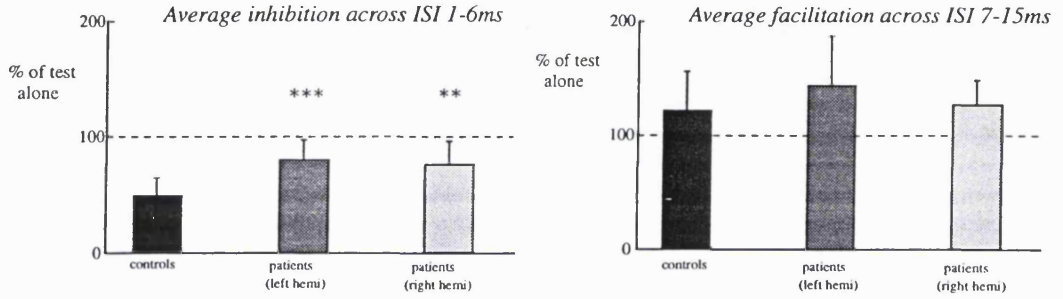


*Fig. 5.2. (Left) illustrates the average level of inhibition across ISIs 1-6 ms in controls and patients (both left and right hemisphere stimulation). The y-axis is the area of the average conditioned response expressed in terms of the area of the average response to the test stimulus alone. There is significantly less inhibition in the patients following either left (MANOVA, p<0.005 ***) or right hemisphere stimulation (MANOVA, p<0.05 **). (Right) illustrates the average facilitation of the test response when preceded by a conditioning stimulus at ISIs 7-15 ms. There is no significant difference between the level of facilitation in controls and patients (either left or right hemisphere stimulation) (MANOVA, p>0.1).*

Discussion

Writer's cramp and Musicians dystonia are examples of task specific focal dystonias. As a result they are believed to be due to dysfunction of the basal ganglia or their connections. The physiological basis of these conditions has been debated for a long time. As outlined in the Introduction, there is evidence of disordered spinal cord and brainstem reflexes in several types of focal dystonia. However, as no pathology has ever been found at either of these sites, it is usually assumed that the changes are due to an alteration in descending inputs from other, higher, structures. Unfortunately, it is unclear which particular structures might be important in relaying these effects. For example, changes in the blink reflex recovery could be due to descending connections between basal ganglia or brainstem, or due to changes in projections to brainstem from cerebral cortex deprived of its normal input

from basal ganglia. Similarly, spinal mechanisms of reciprocal inhibition receive input from many supraspinal structures including both the cortex and brainstem.

The purpose of the present experiments was to investigate the physiology of motor cortical areas in dystonia, since these are known to be one of the primary output targets of the basal ganglia. To do this the technique of transcranial magnetic stimulation over the motor cortex was used. Like others (Thompson *et al.*, 1994) the results show that the threshold for evoking an EMG response in active or relaxed muscle was the same as in normal subjects. In contrast, major changes in excitability became evident when using double pulse testing at short interstimulus intervals.

As described in the Introduction (Chapter 1), this suppression is probably of a cortical nature and reflects activity of local intracortical inhibitory interneurons.

Paired pulse suppression in dystonia

The time-course of paired pulse testing is complex, beginning with a period of pronounced suppression followed by less prominent facilitation. Many circuits, with overlapping time courses could contribute to the effect. The curve demonstrates only that inhibition dominates the early timing whereas facilitation dominates later. Less suppression early in the time-course in the patients with focal dystonia indicates that there is a relative decrease in the excitability of cortical inhibitory systems.

The question is whether this reduction has any relation to the clinical picture of dystonia. As mentioned in the previous chapter, it is interesting to note that in the primate injection of the GABA antagonist bicuculline into the motor cortex results in loss of directional specificity of cortical cells (Matsumura, Sawaguchi and Kubota, 1991) and inappropriate contraction of antagonist muscles during reaction time wrist movements (Matsumura *et al.*, 1991). Therefore, it is suggested, that the reduction in excitability of intracortical inhibitory circuits which is observed in these patients with focal dystonia (the activity of is measured by this paired pulse technique) may be one factor which contributes to the inappropriate and excessive muscle activity which occurs when they attempt fine manipulations. Reduced activity in inhibitory circuits may also explain why PET studies of patients with idiopathic dystonia show a decreased blood flow in primary motor cortex during voluntary joystick movements (Brooks, 1994).

One criticism of the proposed role of cortical inhibition in producing dystonia is that the reduction in paired pulse suppression is seen both in the motor cortex contralateral to the affected hand and in the other hemisphere (although the latter comparison was made between the patients right hemisphere and the controls left hemisphere we have not experienced any major variation in the amount of inhibition in the two hemispheres of normal subjects). However, such bilateral abnormalities in cases of unilateral dystonia (Reilly *et al.*, 1992; Tempel and Perlmutter, 1993) are not an uncommon result. Interestingly, when some patients with Writer's cramp learn to write with their "unaffected" hand, because of the difficulties with their dominant hand, as many as 25% of them go on to develop symptoms in the other hand (Sheehy, Rothwell and Marsden, 1988). This suggests that subclinical abnormalities may have been present bilaterally from the beginning of the disease.

Another apparent problem with the present findings is that there was no significant difference in the level of inhibition when patients with simple and dystonic Writer's cramp were compared, even though the dystonic spasms were worse in the latter group. This may have been because the muscle investigated (FDI) was equally likely to be affected in either group of patients and hence showed a similar lack of cortical inhibition in the two groups. It may be that if the present study had investigated muscles that were affected in patients with dystonic Writer's cramp but not patients with simple Writer's cramp there may have been a difference in the level of inhibition. Unfortunately, it is more difficult to perform these measurements on proximal muscles as the threshold for stimulation usually is much higher than in distal muscles. Also it is important to remember that these recordings were obtained when the subjects were at rest, and had no dystonic symptoms. It is possible that if the patients had been investigated when active a different picture may have emerged.

In conclusion, although it is impossible to be certain, it is likely that these alterations in the excitability of inhibitory systems within the motor cortex play a part in the abnormal movements seen in dystonia.

Four of the patients studied were being treated with botulinum toxin. Botulinum toxin acts principally at the neuromuscular region but may also have an

effect on central structures, either by retrograde transmission up motor axons, or by secondary changes in neural circuitry consequent on altered peripheral input caused by muscle weakness. For example, it has been reported that reciprocal inhibition in patients with Writer's cramp is normalised after botulinum toxin treatment (Priori *et al.*, 1995). However, at the level of the cortex, recent PET studies have failed to show any significant alterations in the level of cortical blood flow following botulinum toxin treatment (Ceballos *et al.*, in press). Similarly, the present results demonstrated no significant difference in the level of cortico-cortical inhibition in those patients who were being treated with botulinum toxin and those who were not. Although only small numbers of subjects were studied, this suggests that botulinum toxin has no effect on the activity of local cortical inhibitory circuits.

Comparison with previous results in patients with Parkinson's disease

In the previous chapter it was shown paired pulse suppression is reduced in patients with Parkinson's disease. Close inspection of the data in the two groups suggests that there may be subtle differences in the form of the suppression in Parkinson's disease and dystonia. The analysis of the time-course revealed a significant interaction term in the case of the Parkinson's disease patients while in the dystonics there was no significant interaction. This suggests that in the two cases the abnormalities of inhibition differ. The latter appear to have reduced suppression at all intervals, whereas the former show more reduction at specific timings (2, 4 and 5 ms). Rothwell *et al.*, (1994 b) showed that when the stimulus parameters were optimized it was possible to demonstrate peaks of short latency facilitation when pairs of threshold magnetic stimuli were delivered. The interstimulus interval that produced this facilitation varied slightly between individuals but needed to be approximately 3 ms. These authors suggested that this facilitation was due to interaction of subthreshold I-wave inputs in the motor cortex. It may be that when inhibition is reduced in the cortex there is a tendency for these I-wave interactions to become more apparent even though the stimulating conditions are not ideal. It is possible that the peaks observed in the Parkinson's disease results are as a result of these interactions. However, it is probably too early to speculate on such differences until larger numbers of patients are studied. It is interesting to note, though, that although the lack of suppression occurs in both these instances of basal ganglia

disease, suppression is normal in patients with cerebellar deficits (Ugawa, Hanajima. Kanazawa, 1994).

Finally, the question arises as to why the decreased cortical inhibition produces excessive muscular activity in patients with dystonia, but not in patients with Parkinson's disease. One possibility is that the movement command itself is reduced in Parkinson's disease (resulting in bradykinesia) and the effects of reduced cortical inhibition are only apparent in the presence of a relatively normal movement command. Hence the effects on cortical inhibition are masked in untreated Parkinson's disease. When the movement command is restored, as during drug-induced dyskinesias, the resulting excess muscle activity becomes clear.

In conclusion, abnormalities of inhibition in the motor cortex of patients with two manifestations of task specific focal dystonia, namely, Writer's cramp and Musicians dystonia, have been demonstrated. These abnormalities are seen bilaterally and are not confined to the motor cortex projecting to the affected limb. Pathology in the basal ganglia may affect inhibition in the bilateral areas of motor cortex but only lead to symptoms with the repeated performance of skilled manipulative tasks.

Chapter 6

ABNORMALITIES OF THE BALANCE BETWEEN INHIBITION AND EXCITATION IN THE MOTOR CORTEX OF PATIENTS WITH CORTICAL MYOCLONUS

Introduction

Cortical myoclonus is thought to involve the sensorimotor cortex and rapidly conducting pyramidal pathways (Kugelberg & Widen 1954; Lhermitte *et al.*, 1971; Pagni *et al.*, 1971; Chauvel *et al.*, 1978; Hallett *et al.*, 1979). It may be elicited by peripheral stimuli or provoked by voluntary action. Such cortical reflex and action myoclonus is most commonly focal or multifocal, but in some patients bilateral or generalised jerks may also occur with stimulation or movement of a single limb (Lance & Adams 1963; Shibasaki *et al.*, 1978; Brown *et al.*, 1991). These more extensive myoclonic jerks have recently been studied, and it was shown that myoclonic activity is able to spread relatively rapidly from an initial focus in one sensorimotor cortex to other ipsilateral cortical areas through cortico-cortical pathways, and to the opposite sensorimotor cortex through the corpus callosum. Brown *et al.* (1991) suggested that similar processes might underlie the generalised seizures common in patients with cortical myoclonus.

Normally the spread of excitation within the cortex and between the two cerebral hemispheres is kept in check by inhibitory processes. The purpose of the present experiments was to test whether transcallosal or corticocortical inhibitory mechanisms (see Chapter 1) function normally in patients with cortical myoclonus. In particular does the amount of inhibition vary between patients with multifocal cortical myoclonus who also have bilateral or generalised jerks, compared to patients with multifocal cortical myoclonus alone, or normal subjects.

Subjects

Patients gave their informed consent to the neurophysiological studies and the procedures were approved by the local ethical committee. Details of the 18 cases studied are presented in Table 6.1. The patients were examined by a neurologist (Dr Peter Brown). The preliminary electrophysiology (EMG recording, back averaging and SEPs) were also performed by the neurologist. These investigations were necessary to catergorise the myoclonus. All the patients had cortical action myoclonus, with a short-latency time-locked cortical correlate preceding jerks during voluntary action. In addition, cases 5, 6, 10, 13, 16, 17 and 18 had cortical reflex myoclonus, defined by giant cortical evoked potentials and C reflexes following

electrical stimulation of the median nerve at the wrist. Cases 3 and 4 had giant cortical evoked potentials in the absence of a reflex EMG response. Cases 10, 11, 14 and 16 have been reported previously (Brown *et al.*, 1991; their cases 6, 1, 9 and 5 respectively).

Nine of the cases were also epileptic, with a history of two or more generalised seizures (see Table 6.1). Fifteen patients were tested while taking various combinations of clonazepam, carbamazepine, phenobarbitone, phenytoin, piracetam, primidone or sodium valproate as shown in the Table. Cases 9, 12 and 18 were investigated off drug treatment. Disability was assessed using the myoclonus disability rating scale (Brown *et al.*, 1993).

Motor threshold to transcranial magnetic stimuli and corticocortical inhibition were measured in 12 healthy subjects, and transcallosal inhibition was measured in 6 healthy subjects. Normal subjects ranged in age from 18 to 75 years. There was no significant change in resting motor threshold, ipsilateral inhibition, or transcallosal inhibition with age amongst normal subjects or patients.

Methods

Motor threshold, corticocortical inhibition and transcallosal inhibition were studied using the techniques described in the General Methods chapter.

Table 6.1 Clinical details

Case	Age and sex	Diagnosis	Cortical reflex myoclonus	Cortical action myoclonus	Seizures	medication daily dose
Non-spreaders						
1	*52M*	*Post-anoxic encephalopathy*	*0*	+	*1/year*	*Clonazepam 2mg Phenobarbitone 30 mg Valporate 800 mg*
2	*18F*	*Progressive myoclonic ataxia*[1]	*0*	+	*1/year*	*Clonazepam 2 mg Valporate 1 g*
3	*30F*	*Progressive myoclonic ataxia (with Histidinaemia)*	*0*[2]	+	*10/year*	*Clonazepam 0.5 mg Piracetam 16.8 g Valporate 2 g*
4	*16M*	*MERRF*	*0*[2]	+	*5/year*	*Clonazepam 1 mg Carbamazepine 800 mg Phenytoin 400 mg*
5	*42M*	*Coeliac disease*	+	+	*0*	*Clonazepam 4.5 mg Piracetam 16.8 g*
6	*16M*	*Progressive myoclonic epilepsy*[1]	+	+	*100/year*	*Carbamazepine 1.2 g Phenytoin 425 mg Valporate 1.5 g*
7	*55F*	*Post-anoxic encephalopathy*	*0*	+	*0*	*Clonazepam 6 mg Piracetam 12 g Valporate 320 mg*
8	*51M*	*Progressive myoclonic ataxia*[1]	*0*	+	*0*[3]	*Clonazepam 1.5 mg Primidone 250 mg Valporate 2.1 g*
9	*53F*	*Post-anoxic myoclonus*	*0*	+	*0*	*None*
10	*36M*	*Progressive myoclonic epilepsy*[1]	+	+	*30/year*	*Carbamazepine 800 mg Clonazepam 3 mg Primidone 250 mg Valporate 3 g*

Spreaders						
11	*77F*	*Post-anoxic encephalopathy*	*0*	+	*0*	*Clonazepam 6 mg Piracetam 14.4 g Valporate 800 mg*
12	*25M*	*Post-anoxic encephalopathy*	*0*	+	*single fit*	*None*
13	*27F*	*Post-anoxic encephalopathy*	+	+	*50/year*	*Clonazepam 4 mg Piracetam 2.4 mg Valporate 1.5 g*
14	*53F*	*Post-anoxic encephalopathy*	*0*	+	*0*	*Clonazepam 4 mg Piracetam 14.4 mg Valporate 2 g*
15	*34F*	*Progressive myoclonic ataxia*[1]	*0*	+	*0*	*Clonazepam 0.5 mg Piracetam 14.4 g Valporate 2 g*
16	*49M*	*Coeliac disease*	+	+	*1/year*	*Clonazepam 12 mg Carbamazepine 800 mg Piracetam 12 g*
17	*14F*	*Sialidosis*	+	+	*0*	*Valporate 800 mg*
18	*68F*	*Coeliac disease*	+	+	*0*	*None*

[1] No underlying cause found despite full investigation. [2]Cases 3 and 4 had giant evoked potentials but no reflex jerks. [3]Total of 6 fits up to age 38, none since

Statistical analysis

Disability scores and motor thresholds are expressed as medians and ranges (Fig.'s 6.2 and 6.3 show that the distributions of these variables were not normal). Differences between disability scores and thresholds were evaluated using the Mann Whitney rank sum test.

The main aim of these experiments was to examine the changes in cortical inhibition in the patients when compared to control subjects. For ipsilateral cortico-cortical inhibition ISIs of 1-6 ms were analysed, as previous work has shown that such ISIs normally have an inhibitory effect on the test response (see Chapter 1 and Chapter 2). For transcallosal inhibition ISIs of 10-14 ms were analysed as, with the stimulus intensities used, these intervals cover the period of maximal inhibition of the test shock in normal subjects (Ferbert *et al.*, 1992). The effect of GROUP (spreaders/non-spreaders), ISI and the interaction between GROUP*ISI were analysed using Multivariate Analysis of Variance (MANOVA). When the Group*ISI interaction was found to be statistically significant, the difference between the groups for each ISI was analysed individually using the Student's t-test. Percent inhibition is expressed as mean $\pm$ standard error of the mean.

Results

Patients were divided into two groups on the basis of the polymyographic findings (Table 6.1). Patients were considered "spreaders" if at least 10% of the action or reflex jerks involved bilateral EMG activity, with the onset of EMG activity in homologous muscles separated by less than 15 ms on the two sides of the body.

In bilateral jerks such EMG activity was only recorded in the stimulated or active limb, and in the homologous muscle on the opposite side. In generalised jerks the same activity was recorded in all four limbs. In "non-spreaders" action, and, if present, reflex jerks nearly always remained confined to the active or moved limb (Brown *et al.* 1991). In these patients less than 5% of jerks involved bilateral EMG activity with the onset of EMG in homologous muscles separated by less than 15 ms on the two sides of the body.

Myoclonic jerks were virtually confined to the stimulated or active limb (multifocal myoclonus) in cases 1 to 10. An example of such an action jerk is shown for case 3 in Fig. 6.1A. Jerks at most involved one limb and adjacent trunk. The cortical spread of excitatory activity responsible for the myoclonus was therefore limited in this group, which were term "non-spreaders" (Brown *et al.*, 1991).

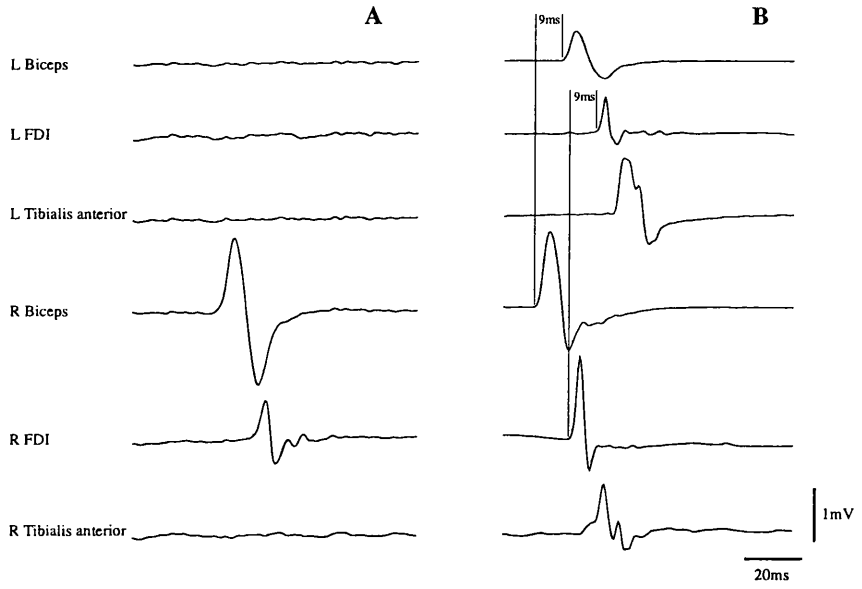


Fig. 6.1: EMG records of a focal action jerk in case 3 (A), and a generalised action jerk in case 14 (B). The jerks are provoked by voluntary movement of the right (R) arm. In (A) myoclonic EMG activity is confined to the active limb (e.g. R biceps and FDI). In (B) EMG activity is generalised, affecting muscles of the right and left (L) arms and legs. There is a latency difference of about 9 ms (see vertical lines) between homologous muscles on the right and left sides of the body, consistent with the transcallosal spread of myoclonic activity from the left to the right motor cortex (see Brown et al. 1991).

Cases 11 to 18 showed frequent additional bilateral (both upper or both lower limbs involved) or generalised (all four limbs involved) action jerks as described by Brown *et al* (1991). An example of a generalised action jerk is shown in Fig. 6.1B for case 14. The reflex jerks in four of these patients (cases 13, 16, 17 and 18) could similarly be extensive. Thus there was evidence of spread of activity across the sensorimotor cortex and between the two cerebral hemispheres. This group were termed "spreaders." The difference in latency of response in forearm extensors or FDI between the two sides of the body was 3 to 10 ms (mean 6 ms) for bilateral action jerks in cases 11 to 18, and 6 to 16 ms (mean 11 ms) for bilateral reflex jerks in

cases 13, 16, 17 and 18. (These differences are similar to those described by Brown *et al.* 1991).

There was no difference between the range of aetiologies or drug treatments between the "non-spreaders" and "spreaders" (Table 6.1). However, "spreaders" were more (p=0.045) disabled than "non-spreaders" (Fig. 6.2).

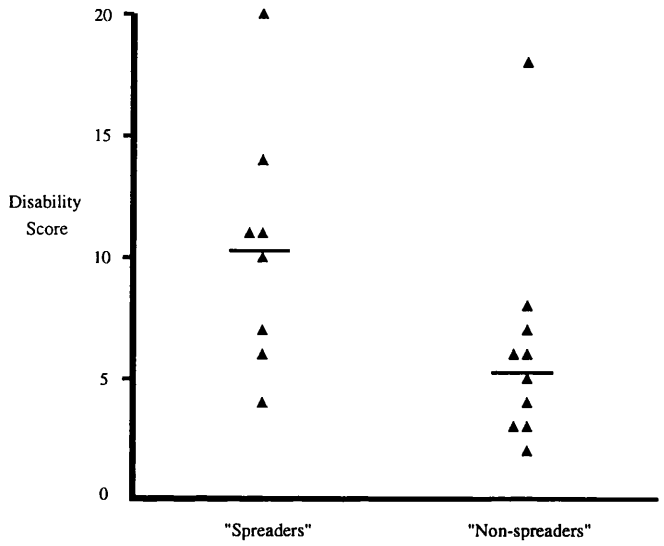


Fig. 6.2. The total disability scores in "spreaders" and "non-spreaders." The median scores are represented by horizontal lines. "Spreaders" were more disabled (p=0.045, Mann Whitney rank sum test).

Motor thresholds to transcutaneous stimulation of the motor cortex.

"Non-spreaders" in general had higher thresholds than either "spreaders" (p=0.023) or healthy controls (p<0.001). The distribution of motor thresholds within the three groups of subjects is shown in Fig. 6.3, in which each tested cerebral hemisphere is plotted.

One striking feature amongst the patients was the number with relaxed motor thresholds in excess of 80% of stimulator output: two "spreaders" and four "non-spreaders" (four and seven tested hemispheres, respectively). The highest motor threshold recorded in healthy subjects was 53%. The pathologically increased thresholds in some of the patients did not simply reflect drug effects: cases 9 and 18 showed greatly elevated thresholds without antimyoclonic treatment (see inverted unfilled triangles in Fig. 6.3). The pathological elevation in motor threshold may be

cortical in origin. In case 18 the relaxed and active thresholds to magnetic stimulation were 85% (cf median 38%, range 28 to 53% in healthy subjects) and 50% (cf median 28%, range 20 to 38% in healthy subjects) respectively. However, when transcranial electrical stimulation of the motor cortex was used in the same subject, motor threshold in active FDI was 40%, which is within the range of active motor threshold in normal subjects (Rothwell *et al.* 1987). Similar findings were evident in Case 8.

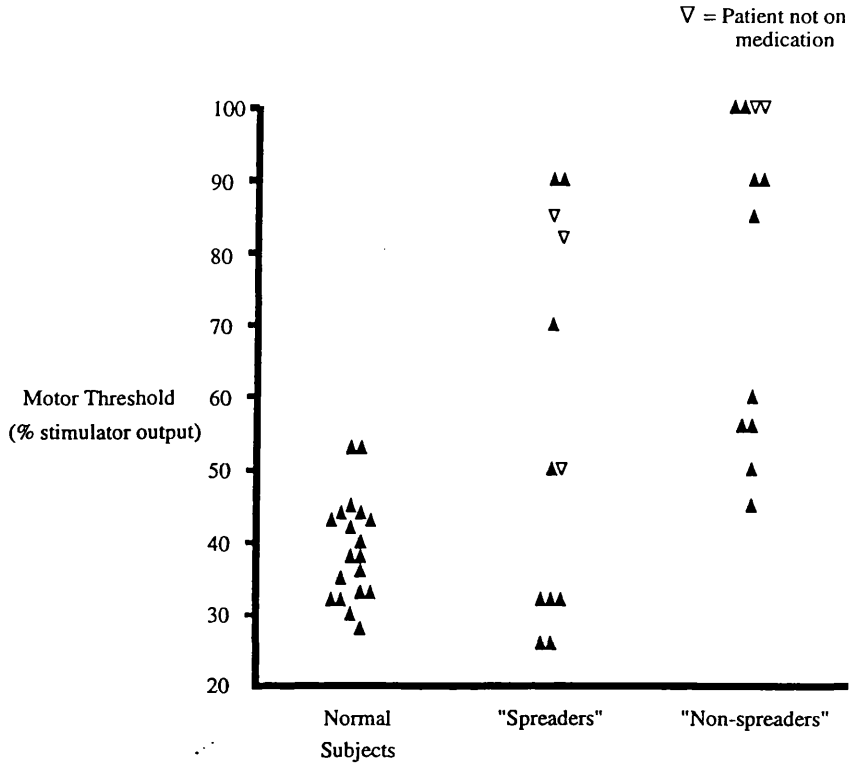


Fig. 6.3: The motor threshold (expressed as the percentage of the maximum output of the stimulator) in FDI at rest to magnetic stimulation of the motor cortex in 12 normal subjects, 8 "spreaders" and 8 "non-spreaders". Each triangle represents a tested hemisphere (in some cases only one hemisphere was tested). "Non-spreaders" had higher thresholds than "spreaders" (p=0.023, Mann Whitney rank sum test) and normal subjects (p<0.001, Mann Whitney rank sum test).

Ipsilateral cortico-cortical inhibition

Ipsilateral cortico-cortical inhibition was measured in cases 1 to 6 and 11 to 16. Thresholds were too high to allow this measurement in all but one of the remaining patients. There was significantly less (MANOVA, $p<0.05$) ipsilateral

inhibition at interstimulus intervals of 1 to 6 ms in the "spreaders" (107±23% of control) compared with the "non-spreaders" (75±15% of control) or healthy subjects (59±10% of control). The difference between "non-spreaders" and healthy subjects did not reach statistical significance. Fig. 6.4A compares the ipsilateral cortico-cortical inhibition in a healthy subject with that in a "spreader" (case 13) at an interstimulus interval of 3 ms.

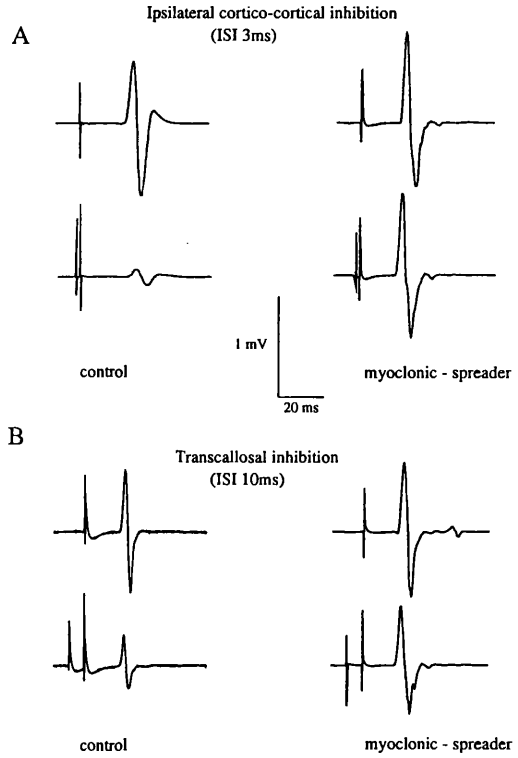


Fig. 6.4. *Raw data of the ipsilateral cortico-cortical inhibition (A) and transcallosal inhibition (B) in a healthy subject and a "spreader" (case 13). In each set of four records the upper trace is the response to the test stimulus alone (single stimulus artefact) and the lower trace is the response to the conditioned test stimulus (double stimulus artefact). (A) There is a marked inhibition of the test shock by a subthreshold conditioning shock delivered over the same hemisphere (ISI 3 ms) in the control subject. No obvious inhibition is seen in the patient. (B) There is marked inhibition of the test shock by a subthreshold conditioning stimulus delivered to the opposite motor cortex (ISI 10 ms) in the control subject. Transcallosal inhibition is very much reduced in the patient.*

The results from all the subjects are summarised in Fig. 6.5. The difference between "spreaders" and "non-spreaders" did not seem to be due to variations in motor threshold as it persisted in those "spreaders" (cases 12 and 14) and "non-spreaders" (cases 2, 3 and 6) with similar motor thresholds (of 45 to 60%). In four "spreaders" (cases 12, 13, 14 and 16), including one patient tested off medication (case 12), inhibition

at conditioning-test intervals of 6 ms or less was replaced by net excitation (Fig. 6.6A). This was seen in only one "non-spreader" (Case 1). There was no correlation between ipsilateral cortico-cortical inhibition and resting motor threshold or the amplitude of the cortical SEP.

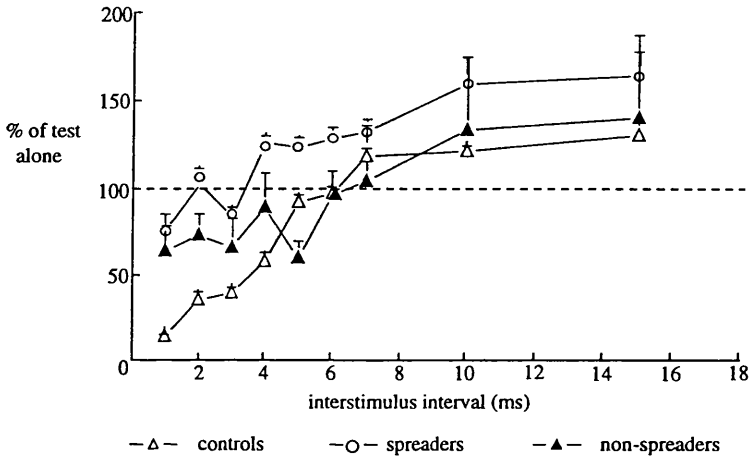


Fig. 6.5: The pattern of cortico-cortical inhibition in 12 healthy controls, 6 "spreaders" and 6 "non-spreaders". The inhibition of the test shock produced by the conditioning shock is expressed as the percentage of the size of the test, when this is delivered alone. In this and ensuing Fig.'s error bars show the standard error of the mean. Cortico-cortical inhibition was reduced in "spreaders" over 1-6 ms (MANOVA, p<0.05) compared to controls and "non-spreaders."

Transcallosal inhibition

Transcallosal inhibition was measured in cases 1 to 3, 6, 7 and 11 to 16. There was significantly less (MANOVA, $p<0.05$) transcallosal inhibition across inhibitory timings (10, 12 and 14 ms) in the "spreaders" (98±6% of control) compared to "non-spreaders" (64±86% of control). Inhibition in the "non-spreaders" was the same as that in healthy subjects (59±6% of control). Fig. 6.4B compares the transcallosal inhibition in a healthy subject with that in a "spreader" (case 13) at an interstimulus interval of 10 ms. The results from all the subjects are summarised in Fig. 6.7. The difference between "spreaders" and "non-spreaders" did not seem to be a threshold effect as it persisted in those "spreaders" (cases 12 and 14) and "non-spreaders" (cases 2, 3 and 6) with similar motor thresholds. Inhibition was replaced by net excitation at intervals of 10, 12 and 14 ms in two patients (Fig. 6.6B), both with bilateral jerks (cases 13 and 14). Case 12, a "spreader", was tested off

medication. Transcallosal inhibition was severely impaired at all intervals below 16 ms (98, 92, 94, 72 and 81% of control at 6, 8, 10, 12 and 14 ms). There was no correlation between resting motor threshold and transcallosal inhibition.

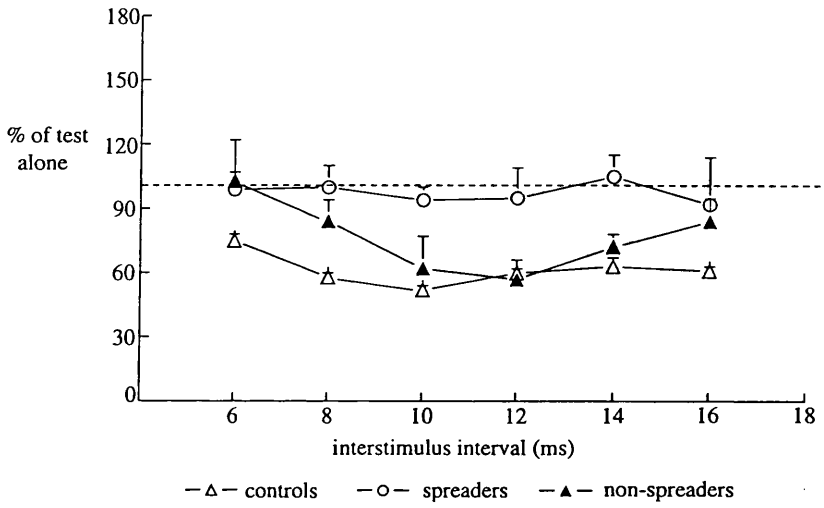


Fig. 6.6: The pattern of transcallosal inhibition in 6 healthy controls, 6 "spreaders" and 5 "non-spreaders". The inhibition of the test shock produced by the conditioning shock is expressed as the percentage of the size of the test, when this is delivered alone. Transcallosal inhibition was reduced in "spreaders" over 10-14 ms (MANOVA, $p<0.05$) compared to controls and "non-spreaders".

Changes in epileptic patients

The patients were also divided into those with epilepsy and those without. Non-epileptic patients had higher motor thresholds (median 85%, range 32-100%) than either epileptic patients (50%, range 26-90%, $p=0.014$), or healthy controls (39%, range 28-53%, $p<0.001$). This elevation of motor threshold in non-epileptic patients was unlikely to be due to drug treatment as all three of the untreated patients were within this group (see Table 6.1). There was no difference in cortico-cortical inhibition (at timings of 1-6 ms) between patients with epilepsy (89±9% of control) and those without (89±14% of control). Similarly, there was no difference in transcallosal inhibition (at timings of 10, 12 and 14 ms) between patients with epilepsy (81±14% of control) and those without (85±8% of control). Strikingly, one patient (case 14) in whom both cortico-cortical and transcallosal were replaced by net excitation had never had a seizure. The results from this case are shown in Fig.6.7.

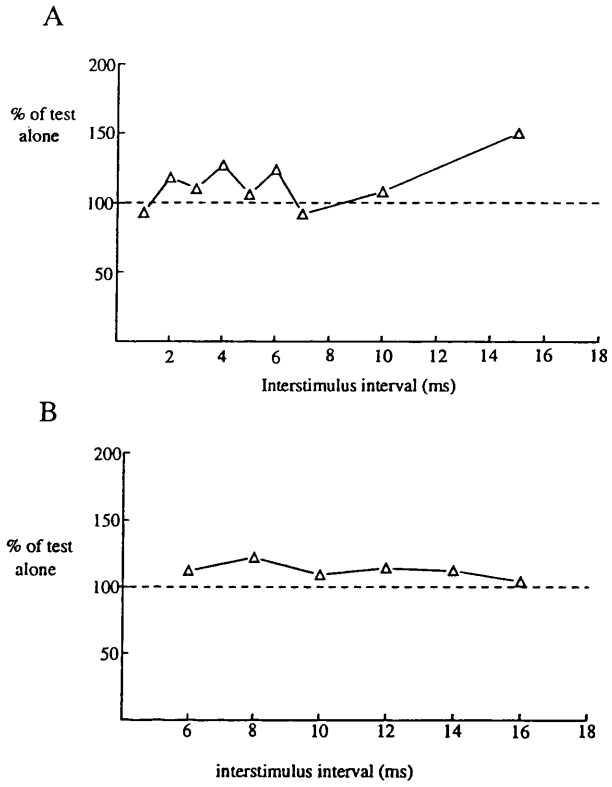


Fig. 6.7: The pattern of cortico-cortical (A) and transcallosal (B) inhibition in case 14. The inhibition normally seen at short latencies has been replaced by net excitation (cf healthy subjects in Fig.'s 6.5 and 6.6).

Discussion

Patients with cortical myoclonus may be divided into those in whom there is frequent spread of myoclonic activity both between and within the sensorimotor cortices, and those in whom there is little spread of myoclonic activity beyond the initial focus. The former have multifocal, bilateral and generalised jerks, whilst the latter have multifocal myoclonus (Brown *et al.* 1991). This broad physiological distinction is of clinical importance as "spreaders" are more disabled than "non-spreaders."

The aim of the present experiments was to define the processes contributing to cortical spread more exactly. Ipsilateral and transcallosal inhibition measured by transcranial magnetic stimulation are reduced in those patients with bilateral or

generalised jerks compared to those in whom myoclonus is strictly multifocal or normal controls. In addition, "spreaders" have lower thresholds to transcranial magnetic stimulation than "non-spreaders." In any one subject the degree of ipsilateral and transcallosal inhibition, and the threshold to transcranial magnetic stimulation will be determined by pathological processes and drug treatment. For example, from experience it appears that antimyoclonic drug treatment may reduce the number of bilateral and generalised jerks, changing a "spreader" to a "non-spreader". However, it seems reasonable to conclude that the general pattern of changes seen here were not solely due to drug effects. The range of drug treatments was broadly similar in the two patient groups. In addition, one "spreader" tested off medication had severely reduced ipsilateral and transcallosal inhibition, and a further two untreated patients had pathologically increased motor thresholds.

Interhemispheric inhibition

Transcallosal inhibition was reduced in "spreaders" between conditioning-test intervals of 10 to 14 ms. These intervals are similar to the interhemispheric delay recorded in the bilateral reflex jerks. Shorter intervals of 3 to 10 ms were recorded in bilateral action jerks, but these measurements are likely to underestimate the true interhemispheric delay (Brown *et al.* 1991). Thus transcallosal inhibition is reduced in these patients with bilateral or generalised jerks during the critical period when excitation reaches the ipsilateral cerebral cortex following stimulation or voluntary movement of one limb.

Intrahemispheric inhibition

Ipsilateral cortico-cortical inhibition was also diminished in "spreaders" relative to "non-spreaders" at conditioning-test intervals of 1 to 6 ms. This would favour the intrahemispheric spread of cortical myoclonic activity known to occur in patients with generalised jerks (Brown *et al.* 1991). A similar deficiency in ipsilateral inhibition has been reported in patients with focal epilepsy (Fong *et al.* 1993).

Thresholds

On the average "non-spreaders" had higher motor thresholds than "spreaders" or healthy subjects. However, individual patients with normal and pathologically

elevated motor thresholds were found in both patient groups. Anticonvulsant medication may elevate motor threshold (Hufnagel *et al.* 1990; Reutens *et al.* 1993). This may account for the higher thresholds in patients relative to healthy subjects, but it seems unlikely that it explains the difference seen between the two similarly treated patient groups. Moreover, two subjects with pathologically high motor thresholds were tested off all medication. Motor threshold was also higher in non-epileptic patients as opposed to epileptic patients or healthy subjects. Reutens *et al.* (1993) have reported that lower thresholds than normal are found in patients with idiopathic generalised epilepsy. Here we have found the corollary, that a pathologically elevated threshold is associated with resistance to seizures and to the cortical spread of myoclonic activity. Such increases in motor threshold may reflect adaptive processes within these patients, aimed at compensating for existing deficiencies of inhibition, including ipsilateral and transcallosal inhibition. These compensatory processes may have been occurring at the level of the cerebral cortex and may depend on transynaptic input to pyramidal neurones. Thus major threshold changes were apparent in 2 patients with magnetic shocks but not with electrical stimuli. At these intensities magnetic stimuli probably produce more I waves than do electrical shocks (Day *et al.* 1989; Berardelli *et al.* 1990).

A series of corticospinal discharges follows magnetic stimulation of the motor cortex. The mechanism behind these discharges is still unknown (see Introduction), but is believed by many to represent the effects of different synaptic inputs on to cortical pyramidal neurones, rather than the autonomous multiple firing of the latter. The pattern of individual corticospinal volleys varies with modality, intensity and orientation of cortical stimulation (Day *et al.* 1989). It seems likely, therefore, that the motor threshold to magnetic stimulation is partly determined by the balance between *tonic* inhibitory and excitatory inputs to pyramidal neurones. Pathological changes in patients with idiopathic generalised epilepsy (Reutens *et al.* 1993) reset these tonic inputs to increase overall excitability, as reflected in lower motor thresholds to magnetic stimulation. Conversely, compensatory physiological processes and anticonvulsant drugs can change the balance in favour of decreased excitability, as reflected in the higher motor thresholds in some of the patients studied here.

The difference in threshold between the different patient groups necessitates caution in the interpretation of the inhibition results. It is possible that motor threshold changes without commensurate change in the threshold for inhibition with a conditioning magnetic shock. If this were the case then the differing intensity of stimulation between "spreaders" and "non-spreaders" might be important. However, this is unlikely to account for the increased cortico-cortical inhibition in the "non-spreaders" as these patients were tested at higher stimulus intensities (had higher thresholds) and cortico-cortical inhibition decreases as stimulus intensity is increased (Kujirai *et al.* 1993). In addition the difference persisted when those individual patients with similar thresholds from the two groups were considered. Transcallosal inhibition does tend to increase with increasing stimulus intensity, but differences were again preserved in patients with similar thresholds.

Epilepsy

It has previously been speculated that the abnormal spread of excitatory activity within and between the two sensorimotor cortices may contribute to seizure generalisation in patients with cortical myoclonus (Brown *et al.* 1991). The findings of the present experiments support the conclusion that there is no difference between cortico-cortical or transcallosal inhibition between patients with cortical myoclonus and generalised epilepsy, and those with cortical myoclonus but no epilepsy. It therefore seems unlikely that deficiencies in these particular inhibitory processes within the sensorimotor cortex play a major part in the epileptic process. Instead, the present finding suggests that impairments of corticocortical and transcallosal inhibition (as measured by transcranial magnetic stimulation) may be epileptic epiphenomena, much more closely associated with the presence and extent of myoclonus. This is particularly striking in those patients in whom early corticocortical inhibition was replaced by excitation. One of these, case 14, had never had a seizure, and a further case (12) only ever had a single seizure. Similarly, transcallosal inhibition was replaced by excitation in two patients, one of whom was not epileptic (case 12).

The normal motor cortex is not concerned with holding epileptic processes in check, but in orchestrating movement, and it is likely that corticocortical and transcallosal inhibition normally act to transform elemental mass movements as seen

in cortical myoclonus in to a meaningful pattern of synergistic activities. Given this perspective it is easier to understand the relationship between the present findings and other instances of deficient inhibition. Previous chapters have examined cortical inhibition in patients with other disorders of movement and have demonstrated abnormalities in Parkinson's disease and Writer's cramp. In both of these conditions there is a deficiency of motor cortical inhibition, but neither disease causes seizures. Results from animal experiments with local injections of bicuculline (Matsumura *et al.* 19912) suggest that one role of inhibitory connections within the motor cortex is to focus activity onto appropriate groups of neurones. Therefore, it seems probable that under normal conditions the role of this inhibition is to "set" the motor cortex so that upon receiving the movement command from higher centres the appropriate output is produced, and inappropriate movement is suppressed.

In conclusion, both transcallosal and ipsilateral cortical inhibition are reduced in those patients with cortical myoclonus who have more extensive jerks. This lack of inhibition will facilitate the transcallosal and cortical spread of myoclonic activity responsible for bilateral and generalised myoclonic jerks. However, deficiencies in transcallosal and cortico-cortical inhibition within the sensorimotor areas of the cerebral cortex are not major factors determining the presence or absence of generalised seizures in patients with cortical myoclonus. In contrast, pathological elevation of the motor threshold to magnetic stimulation is associated with a lack of spread of excitatory myoclonic activity and an absence of seizures.

Chapter 7

MOTOR CORTEX REORGANISATION IN MAN

Introduction

As discussed in Chapter 1 there is a vast literature on the subject of cortical reorganisation following deafferentation in animals. Recently, similar phenomena have been investigated in man by the use of transcranial magnetic stimulation and altered corticospinal excitability has been demonstrated. It has been suggested that these findings in animals and humans represent plasticity in the central nervous system and that modulation of GABAergic activity within the cortex might be one mechanism by which alterations in the cortical representation, particularly in the short term, are brought about. If the inhibition recorded using the technique of corticocortical inhibition reflects activity of intrinsic inhibitory (possibly GABAergic) interneurons, it is possible that alterations in cortico-cortical inhibition would accompany changes in cortical representation. The experiments described in this Chapter were designed initially to study the relationship between cortical inhibition and cortical reorganisation. However, they lead to two new observations on the nature of motor cortical plasticity and it is these that are detailed here.

Methods

Subjects

Local ethical permission was obtained for the experiments described in this section and all subjects gave informed consent.

Traumatic amputees

Four traumatic amputees were investigated (for clinical details see Table 7.1). These subjects were recruited for the study by Dr John Kew from the MRC PET unit at the Hammersmith Hospital. EMG responses following transcranial magnetic stimulation were recorded in either the forearm flexor (FF), deltoid (Del), or trapezius (Trap) muscles depending upon the level of amputation (see Table 7.2). All four subjects were studied using the cortical mapping technique. Two of the amputees (subjects 1 and 2) also had intensity curve data recorded. Data were recorded in both the relaxed and active conditions.

Subject	Age (years)	Time since amputation (years)	Muscle tested	Level of amputation
1	48	7	Delt	R. upper arm
2	45	9	FF	R. lower arm
3	52	13	FF	R. lower arm
4	53	31	Trap	R. upper arm

Table 7.1. Clinical details for traumatic amputees.
Abbreviations used in Table 7.1. Delt = deltoid, FF = forearm flexors, Trap = trapezius.

Normal subjects

Six neurologically normal subjects were studied before, during and after a period of ischaemia of the left hand lasting up to 1 hour. The ischaemia was induced by inflating a blood pressure cuff around the wrist above systolic blood pressure.

Two of the normal subjects were studied using the cortical mapping technique. Recordings were made before the start of ischaemia (but with cuff in place) and 40 minutes into the ischaemic period. Responses were made from the left biceps muscle in both the relaxed and active conditions.

In all six subjects intensity curves were recorded for both the right and left biceps muscle following contralateral cortical stimulation. Recordings were taken with the target muscle relaxed and while maintaining a minimal (10% MVC) voluntary contraction. The right biceps muscle acted as a control for any general increase in cortical excitability (possibly as a result of anxiety). Intensity curve data was recorded at four different times; before the start of ischaemia, 30 minutes into ischaemia, 50 minutes into ischaemia, and 30 minutes following deflation of the cuff (by which time all subjects reported the hand to be feeling completely normal). Sensory and motor disturbances associated with the ischaemia generally disappeared within several minutes of cuff deflation and no subjects reported any ill effects.

Five further normal subjects were studied. Each of these subjects had several episodes of ischaemia and were studied using the techniques described above. Individual subjects were not always investigated with the same technique on

different occasions. This was the case because differences in response to ischaemia on repeated exposure only came to light when we had completed the experiments.

Techniques

Cortical mapping

In order to achieve an indication of the cortical representation of a muscle magnetic stimuli were applied to a variety of scalp sites and the responses in the target muscle recorded. A grid of sites was marked on the scalp surface with points separated by 1 or 2 cm in both the anterior/posterior and medial/lateral directions. Three stimuli were applied at each scalp site in a random manner. Response maps were constructed with the target muscle in both the relaxed and active (10% MVC) states. To perform the mapping stimulus intensity was set to 20% (of threshold) above relaxed threshold for the relaxed map, and 20% (of threshold) above active threshold for the active map. A figure of eight coil (as described earlier) was used in an anterior/posterior orientation (current in the coil flowing posteriorly) to stimulate at each cortical site. The coil was positioned at each scalp site as described earlier i.e. the anterior edge of the wing intersection is placed over the scalp site. The average size of the responses evoked at each scalp site are graphically expressed in the form of a three dimensional map.

Intensity curves

Magnetic stimuli, using a figure of eight coil, were applied at the optimal scalp site for evoking responses in the target muscle at incremental intensities. Five stimuli were given at each intensity and the average response size calculated. Responses were recorded following stimulation of both hemispheres. Intensities ranged from 20-90% (of stimulator output) and responses were recorded both with the target muscle relaxed and also during a minimal tonic contraction. Audiovisual feedback was given to assist the subjects maintain the correct level of contraction.

In the following results section response sizes are described in terms of either peak to peak size of area of response.

Statistics

In the amputees threshold differences between the hemispheres were analysed using paired Student's t-tests. Comparisons were made with both the target muscle relaxed and active. In both the amputees and normals with ischaemic blocks the number of active sites was analysed using paired Student's t-tests. Again comparisons were made in both the active and relaxed conditions. In the control subjects intensity curve measurements were taken before during ischaemia in both the active and relaxed conditions. The difference in the response amplitudes at various intensities and durations of ischaemia were analysed using multiple analysis of variance (MANOVA).

Results

Amputees

Thresholds

In the four traumatic amputees studied there was no significant difference between the right and left hemisphere thresholds in either the relaxed or active conditions (see Table 7.2). In the relaxed condition the right hemisphere threshold was 53±17% (mean±SD) and the left hemisphere threshold was 55±19% ($p>0.1$, paired t-test). In the active condition the right hemisphere threshold was 44±12% and the left hemisphere threshold was 43±15% ($p>0.1$, paired t-test).

Mapping experiments

When the target muscle was relaxed there were significantly more scalp sites from which an EMG response could be evoked when stimulating the hemisphere contralateral to the amputation (left) (12.75±4) than following stimulation of the right hemisphere (8.25±2) ($p<0.05$, paired t-test). When the target muscle was voluntarily activated this picture was reversed with the left hemisphere having less (7.75±5) excitable sites than the right hemisphere (13.5±7) (Table 7.2). However, this difference was not significant ($p>0.1$, paired t-test). Fig. 7.1 illustrates, for one subject, the above findings. In the relaxed condition, with stimulation at the optimal site, the responses were much larger when stimulating the left hemisphere (421±239% of right hemisphere stimulation). In every subject the mean response

was larger following left hemisphere stimulation. However, this difference was not significant and probably reflected the small sample size and the variability in the size of the effect. During voluntary contraction the responses were significantly ($p<0.05$, paired t-test) smaller following left hemisphere (72±17%) stimulation than right.

Subject	Muscle	Threshold (% stim output)				Size (% of control)				No. of active sites			
		Relaxed		Active		Relaxed		Active		Relaxed		Active	
		R	L	R	L	R	L	R	L	R	L	R	L
1	Delt	73	80	55	60	100	237	100	48	8	14	8	3
2	FF	38	40	30	30	100	792	100	88	6	10	10	4
3	FF	40	38	30	34	100	512	100	81	8	9	13	13
4	Trap	60	60	53	50	100	141	100	69	11	18	23	11

Table 7.2. Data from mapping experiments on traumatic amputees. The average area of the evoked response was calculated following stimulation at the optimal site with a stimulus intensity of 20% above either relaxed or active threshold (as described in Chapter 2).Control refers to the size of the contralateral response evoked by right ("intact") hemisphere stimulation. Abbreviations used in Table 7.2. Delt = deltoid, FF = forearm flexor, Trap = trapezius, R = right hemisphere stim, L = left hemisphere stim.

Intensity curves

Two of the amputees (MM and PB) had intensity curve data recorded. In both subjects the curve was steeper (larger responses at a given stimulus intensity) when stimulating the hemisphere contralateral to the amputation. This difference was seen with both the target muscle relaxed or active. Data from one of these subjects (MM) is illustrated in Fig. 7.2.

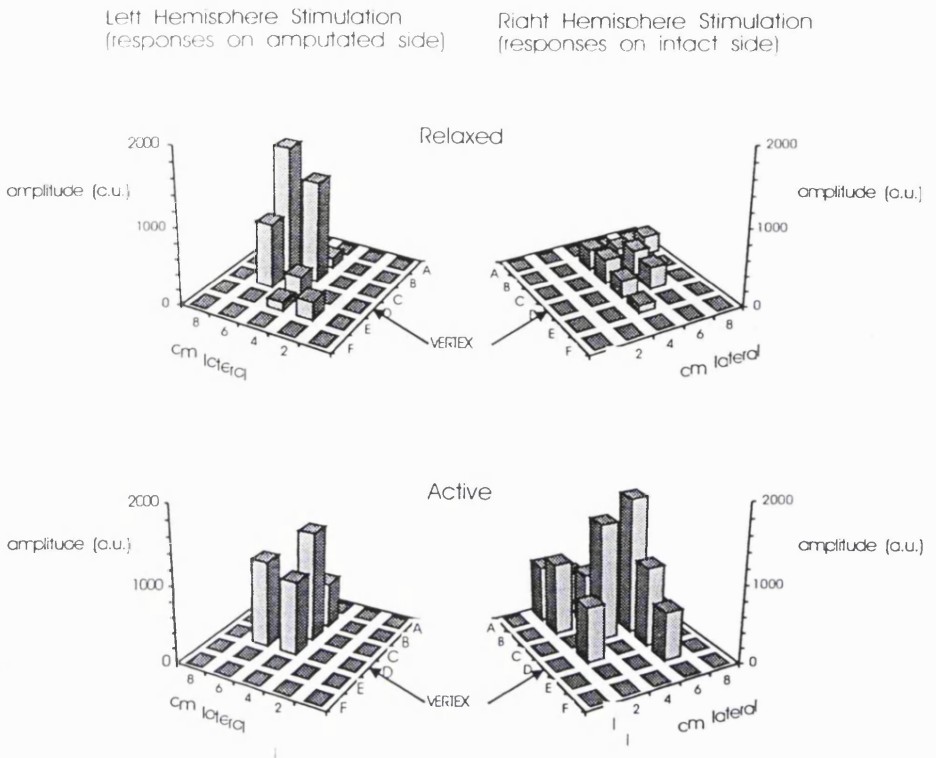


Fig. 7.1. Three dimensional motor map from traumatic amputee (subject 3). This subject had a low right forearm amputation and responses were recorded from the forearm flexor muscles bilaterally in the relaxed (top) and active (bottom) conditions. The height of each histogram indicates the mean amplitude of three responses evoked by stimulation at that scalp location. The scalp sites are marked out in a grid with 2 cm divisions in the both the anterior-posterior and medial-lateral directions. Responses in the relaxed condition are much larger following stimulation of the left hemisphere. In the active condition responses are of a similar size following stimulation of both hemispheres.

Ischaemia experiments in normals

Mapping experiments

Two subjects had mapping experiments performed before and during a period of left hand ischaemia. The mapping was performed at approximately 40 minutes into ischaemia at which time there was complete paralysis of intrinsic hand muscles. With the target muscle (left biceps) relaxed both subjects showed an increase in the number of sites from which responses could be evoked. In the first subject there were 8 excitable sites before ischaemia and 14 when tested during ischaemia. The second subject showed a smaller increase with 7 sites excitable before ischaemia and 8 during ischaemia. During voluntary contraction, there were the same number of active sites during ischaemia as there were in the pre-ischaemia

period (one subject having 10 excitable sites, and the second subject having 6). For the two subjects in the relaxed condition the average responses during ischaemia were 326% of the preischaemia control (range 242-426%) in the relaxed condition.

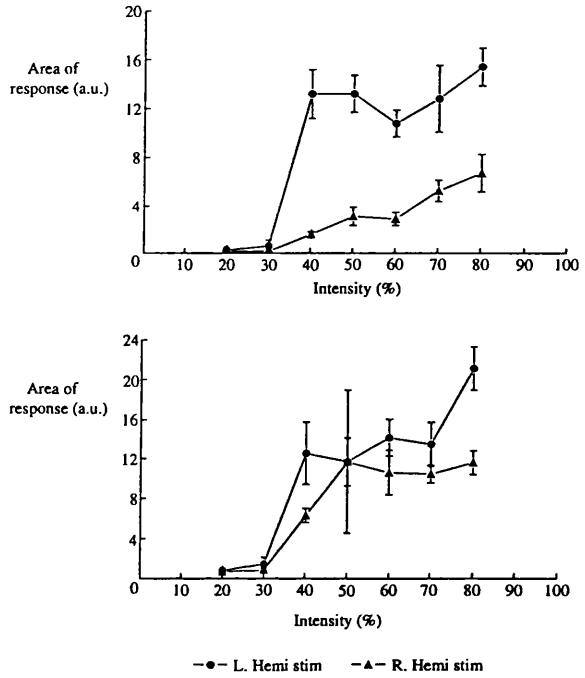


Fig. 7.2. Intensity curves recorded in a traumatic amputee (subject 2). Each point on the curve represents the mean area of the response evoked by 5 stimuli to the optimal scalp location (the bars indicate ±1 SD). Responses were recorded in both the relaxed (top) and active (bottom) conditions. When relaxed, responses following left hemisphere stimulation are much larger than following right hemisphere stimulation. With the target muscle active there is little difference in the curves. Note that there is no obvious difference in threshold between the hemispheres.

When active the responses during ischaemia were only 142% (range 73-334%) of the preischaemia control. Fig. 7.3 shows three dimensional motor maps from one of the normal subjects prior to and during a period of ischaemia.

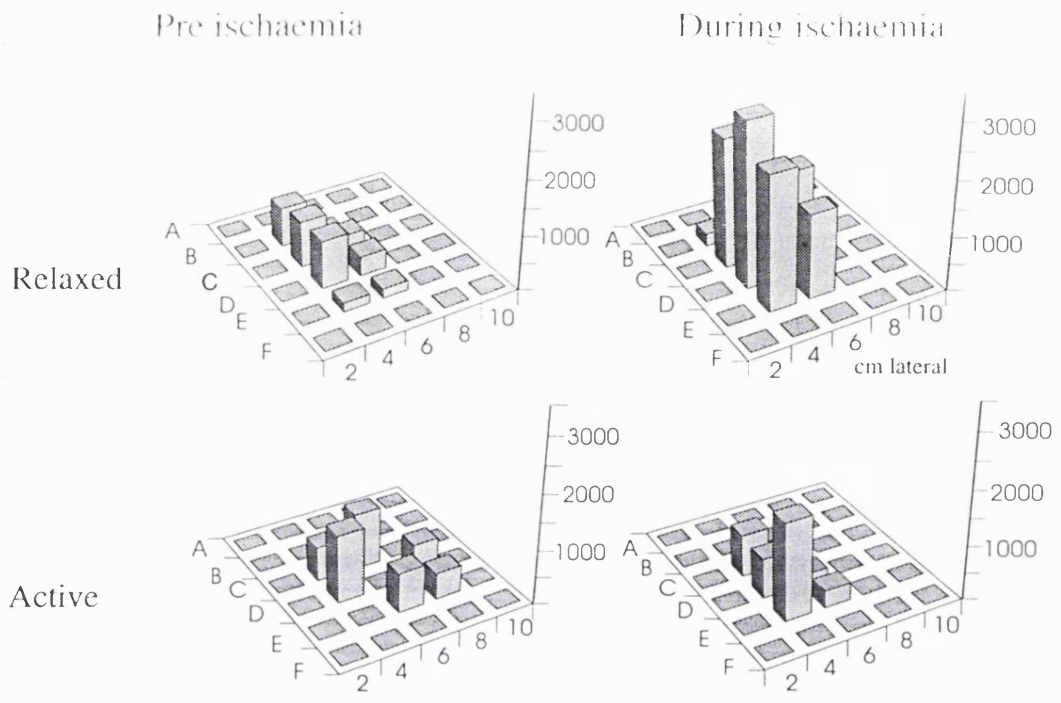


Fig. 7.3. Three dimensional motor maps from a normal subject prior to and during a period of left hand ischaemia. Responses were recorded from the relaxed and active left biceps muscle. It can clearly be seen that when the target muscle is relaxed the responses are much larger during the period of ischaemia than before the start of the ischaemia.

Fig. 7.4 shows representative responses (evoked from the optimal scalp site) from the same subject that were recorded prior to and during a period of ischaemia. It can be seen that when the target muscle was relaxed responses recorded during ischaemia were much larger than those seen prior to the ischaemic period. Responses recorded with a minimal voluntary contraction were of similar size under both conditions.

Intensity curves

Six control subjects had intensity curve data recorded during a period of ischaemia. Recordings were taken 30 minutes and 50 minutes into the period of ischaemia and 30 minutes following the deflation of the cuff. During the period of ischaemia thresholds were not determined accurately but from the individual subject intensity curve data it can be seen that no major alteration in threshold was apparent (see Fig. 7.5). Due to the small number of subjects investigated in this set of experiments we were unable to test the significance of the changes at every

intensity level. In order to analyse the data it was grouped in the following way; intensities of 30,40,50% and 60,70,80%.

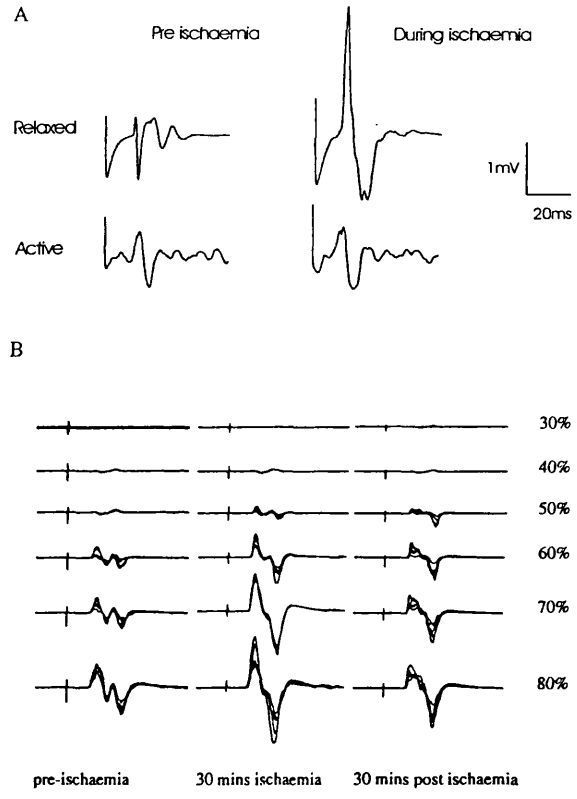


Fig. 7.4. (A) Raw data traces from one subject before and during a period of left hand ischaemia. Responses are recorded from the left biceps muscle. During ischaemia responses in the relaxed condition increase in size dramatically while there is little change in the size of responses evoked during a minimal background contraction. (B) raw data from one subject prior to, during (30 mins), and after (30 mins) a period of ischaemia. Responses are recorded in the relaxed biceps muscle following cortical stimulation at a range of stimulus intensities (expressed as a percentage of the maximal stimulator output). Again, during ischaemia the responses are of much greater amplitude than during either the pre or post ischaemic periods.

The data was then analysed using multiple analysis of variance (MANOVA). Data obtained with higher levels of stimulation (90 and 100%) were not included in the analysis as several of the subjects were not investigated using these high levels of stimulation (for reasons of subject comfort). With the muscle at rest, responses from the left biceps were significantly larger during than when compared to the baseline values obtained before the start of ischaemia. This increase in response amplitude was seen at both 30 minutes (MANOVA, $p<0.05$) and 50 minutes (MANOVA, $p<0.05$). As can be seen from Fig. 7.5 this results in the intensity curve being steeper

during ischaemia than in the control condition. The response curve seen with 50 minutes of ischaemia is very similar to that seen with voluntary contraction prior to the ischaemia. The amplitudes of the responses in the left biceps muscle were not significantly different from control when recorded 30 minutes after the deflation of the cuff (MANOVA, $p>0.1$). The amplitudes of responses recorded in the right arm were not significantly larger during the ischaemic period than during the control (e.g. at 50 mins of ischaemia; MANOVA, $p>0.1$: see also Fig. 7.5C).

Intensity curves obtained with the left biceps muscle voluntarily activated were not significantly different during the ischaemia when compared to the pre-ischaemia active controls (MANOVA, $p<0.5$)(Fig. 7.5B).

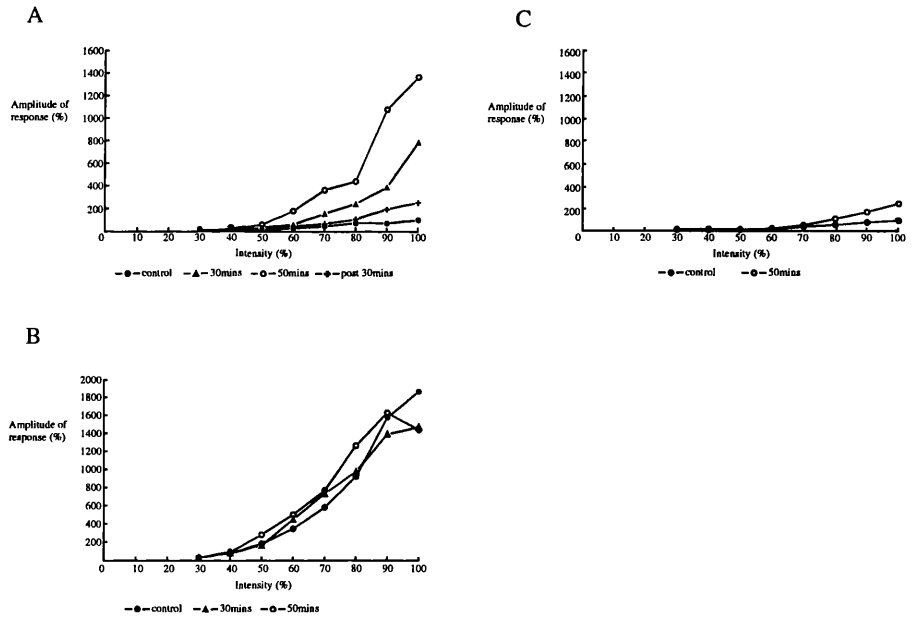


Fig. 7.5. Intensity curve data recorded in normal subjects before during and after a period of left hand ischaemia. Each point represents the average response amplitude (% of maximum control response in the relaxed condition) from six subjects. Responses are recorded in the left biceps muscle. (A) In the relaxed condition during ischaemia there is a steady increase in response size. At the end of the period of ischaemia (50 minutes) there is a ten-fold increase in response size. Within 30 minutes of cessation of the ischaemia response size has almost returned to control levels. (B) When the target muscle was active there was little change in the response amplitude during ischaemia.(C) data recorded in the control (non-ischaemic) right arm. There is only a small non-significant increase in the size of the response during ischaemia.

Repeated testing in relaxed muscle

Five subjects were tested following more than one period of ischaemia. The period between these periods of ischaemia ranged from several weeks to approximately two years. The testing protocols on the separate occasions were not standardised. This was because it was not until the data was studied retrospectively that it became apparent that there were differences in the results with repeated ischaemic episodes. In all cases the target muscle was the biceps muscle. Following the first period of ischaemia all subjects showed a clear increase in size of the target muscle responses relaxed. However, after successive periods of ischaemia the effect was reduced or even abolished. Examples from two of the subjects are shown in Fig.'s 7.6 and 7.7.

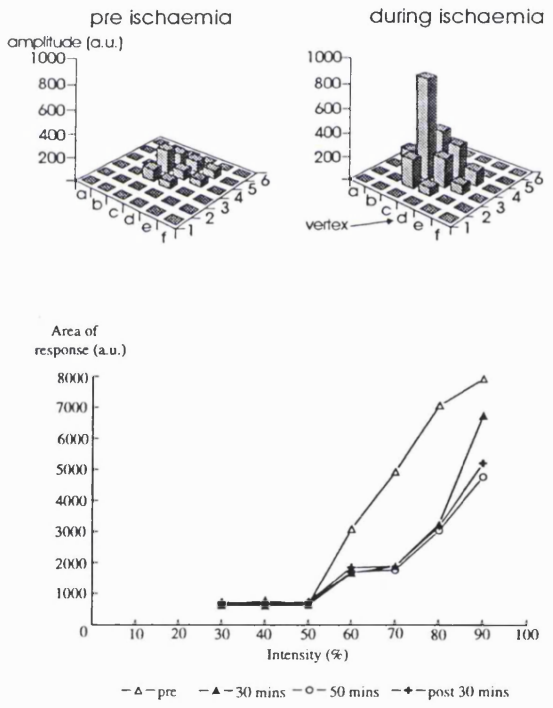


Fig. 7.6. Data from one subject obtained following two periods of ischaemia. In (A) cortical mapping was performed during a period of left hand ischaemia. Responses were obtained in the relaxed left biceps muscle. Responses were of much greater size, and were obtainable from more scalp sites, during ischaemia than in the control (pre-ischaemia) condition. Intensity curves obtained in the same subject during a second ischaemia experiment are shown in (B). Again, responses are obtained in the relaxed left biceps muscle during a period of left hand ischaemia. On this occasion responses recorded during ischaemia were no larger during ischaemia than in the control condition.

Fig. 7.6 illustrates data recorded in one subject following ischaemic periods which were separated by more than two years. On the first occasion cortical mapping was performed and it can be seen that the responses recorded (with the target muscle relaxed) during ischaemia on this occasion were much larger during the ischaemia than they were during the control period. When the subject was tested on the second occasion (using intensity curve measurements) there was no increase in size of the responses seen during ischaemia. Fig. 7.7 illustrates the results obtained in another subject. Two intensity curve experiments were performed following periods of ischaemia separated by approximately three weeks. During the first episode of ischaemia there was clear enhancement of responses in the relaxed biceps muscle. At an intensity of 80% responses were 266% of control when tested 50 minutes into ischaemia. When tested during the second occasion there was less enhancement of responses during the ischaemia than on the first occasion. At an intensity of 80% the responses were only 140% of control.

Analysis of this data is slightly problematical because of the differences in techniques used to study the subjects. In order to compare mapping and intensity curve data comparisons were made of the effect of ischaemia on the size of responses evoked from the optimal stimulation point (i.e. one of the many mapping points that were stimulated) at an intensity of 20% above threshold (i.e. one of the many intensities used during intensity curve measurements). Using this data there is a clear reduction in the effect on the two occasions. On the first occasion there was an average increase in response size during ischaemia of 120±55%, while on the second occasion there was an average reduction of 9±44% during ischaemia (Student's paired t-test $p<0.01$).

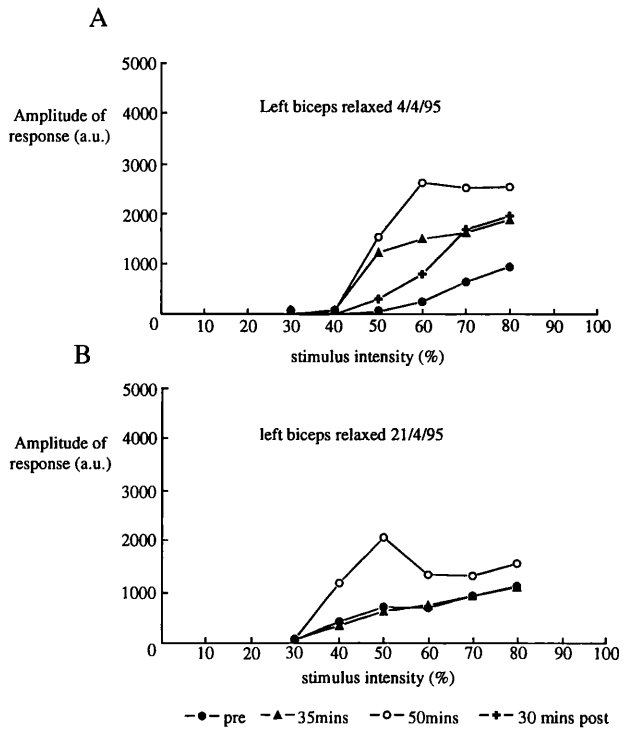


Fig. 7.7. Intensity curves recorded in one subject following periods of left hand ischaemia lasting approximately 60 minutes. Responses are recorded from the relaxed left biceps muscle. On the first occasion (4/4/95) there is a progressive increase in response size during the ischaemic period. Following release of the cuff there is a trend back towards the control values (30 mins post). When tested following a second period of ischaemia approximately 2 weeks later there is a less clear increase in response size during the ischaemic period. (Note; on the second occasion recordings were not made following the end of the ischaemic period).

Discussion

There are three main findings from the experiments described in this chapter. Firstly, the results have confirmed previous reports of alterations in cortico-spinal excitability with changes in afferent input. Intensity curve measurements yielded similar results to those obtained with the mapping experiments. These experiments yielded no information as to the level within the corticospinal system that these changes take place but it has been suggested previously that much of the change takes place at a cortical level (Fuhr *et al.*, 1992). Secondly, the changes seen in cortico-spinal excitability seen when the target muscle was relaxed are not apparent when the muscle is voluntarily activated. Thirdly, when subjects are tested on repeated occasions the changes observed in the relaxed state become less marked or absent.

Comparison of mapping and intensity curves

When a magnetic stimulation map is constructed the position of the centre of the map probably corresponds quite well to the expected location of the optimal stimulation site on the somatotopic map of the precentral gyrus (Wasserman *et al.*, 1992a). Hence mapping allows an estimation of the site of representation of different muscles on the cortical surface to be determined. However, the extent of the map does not necessarily correspond to the anatomical extent of the corticospinal projection to a given muscle since the area of a magnetic stimulation map is a function of the stimulus intensity - greater intensities resulting in larger maps. Even using a constant stimulus intensity, changes in the area of a map can be caused either by an increased anatomical map or by an increase in the excitability of a constant sized projection area. Thus these maps cannot distinguish between changes in the anatomical extent of cortical representations and alterations in its excitability. Intensity curves are influenced by the same factors. If the excitability of the projection area increases then the intensity curve will be steeper, if the excitability decreases then the curve will be shallower. These measurements will also be affected by changes in the extent of the anatomical map. Therefore I would like to suggest that both magnetic stimulation maps and intensity curves can be used to examine the area and excitability of cortical representations but are unable to distinguish between an alterations in one or other variable. For several reasons it may often be more appropriate to use intensity curve recording rather than mapping techniques. Firstly, it is far quicker to record intensity curve data than mapping data. This is important where time is at a premium, for example during ischaemia experiments. Secondly, there is less potential for compounding response variability by constant coil movement which is necessary for the mapping experiments.

Findings of present experiments

There are two main points to be made from the experiments described in this chapter. The first of these is the effect of voluntary contraction on alterations seen in cortical excitability following deafferentation and the second concerns the effect of repeated testing.

Effect of voluntary contraction.

The results in relaxed muscle have confirmed the findings of previous groups who have used magnetic stimulation to demonstrate map expansion proximal to ischaemic or anaesthetic blocks (Brasil-Neto et al 1992), amputation (Cohen *et al.*, 1991). In this set of experiments the level at which these changes take place has not been investigated but other data suggests that most of the change takes place at the level of the cortex (Fuhr *et al.*, 1992). The mechanism behind the increase in excitability seen in the relaxed state remains unclear. However, it is known that the motor cortex receives abundant sensory feedback from the periphery (Rosen and Asanuma, 1972). Sensory input appears to be vital in maintaining cortical representations in S1 (Hubel and Wiesel, 1970; Kaas *et al.*, 1983). There is a close link between sensory input and motor output to similar body areas and it may be a mismatch in input and output that drives the reorganisation. In animal experiments changes in cortical organisation can take place within a very short space of time. In adult rats for example, within 1-2 hours of a nerve lesion responses can be seen in new muscle groups from stimulation within the dennervated cortex (Donoghue *et al.*, 1990). Following repetitive intracortical stimulation within M1 the cortical representation of that part has increased in size within 30 minutes (Nudo *et al.*, 1990). It is probable that these alterations in cortical organisation and excitability which happen over such a short time scale must be brought about by alterations in synaptic efficacy i.e. unmasking of existing synaptic connections. The changes in excitability observed in the relaxed condition, following a period of ischaemia, occur over a similar time-scale. It appears likely, therefore, that they are due to mechanisms that rely on changes in synaptic efficacy.

Quite unexpectedly the effect of ischaemia was much less or absent when measurements were made during voluntary contraction of the target muscle. It is possible that this finding may have been an artefact due to, for example, differences in the size of the evoked responses in the two cases. However this appears unlikely as the effect is seen across a wide range of response amplitudes (see intensity curves). It therefore appears as if there is a fundamental difference in the effect of deafferentation in the two conditions.

Afferent input changes during movement and it is possible that this has an influence on cortical excitability. The contribution from this mechanism is unknown.

Physiological significance of results in active and relaxed muscle

It has been proposed that the alterations seen in the cortical representation following deafferentation may have a useful role to play in recovery from the insult and that the expanded cortical representation of the remaining muscles would lead to an improvement in function. However, if this were true, then we might expect that these changes would be apparent in the physiologically important state i.e. during voluntary movement. Since this was not the case, the functional benefit of this reorganisation at rest becomes difficult to understand. It is possible that this increased excitability at rest would make the cortical projection to a muscle more accessible to a motor command which may lead to it being easier to make voluntary movements. However, no evidence exists to support this theory.

I propose that, in normals, when relaxed the motor cortex is actively controlled by inhibitory input. This inhibition would stop unwanted movements. During a movement this inhibition is "turned down" thus opening up the projection to the muscles necessary and therefore facilitating the intended movement. When a muscle or group of muscles are unavailable for use, due to amputation for example, then there would be no benefit in inhibiting the cortical cells that project to the muscles that are no longer present. This would result in the level of cortical excitability being similar in the relaxed and active states. If this is the case then in terms of the testing procedures used we would see much larger responses in the deafferented limb than in the control limb when the subject was at "rest". However, during activity there would be little difference in the normal and deafferented limb.

Repeated testing

The second finding of these experiments is that on repeated testing the alterations seen in cortical excitability are not as obvious and may even disappear. There may be many months (in one subject greater than 2 years) between the ischaemically induced deafferentation episodes. The reason for this remains unclear but it suggests that the "reorganisation" in normals seen over short time periods is quite different to that seen in traumatic amputees where changes in cortical excitability are still apparent many years after the amputation. There is evidence of new synaptic growth in the central nervous systems of adult animals following deafferentation (Raisman and Feld, 1973) and this mechanism may have a role to

play in the changes observed in the amputees. This finding casts further doubt on any functional significance or benefits that might be proposed for these short term changes in cortico-spinal excitability.

Chapter 8

GENERAL DISCUSSION

The technique of transcranial magnetic stimulation (TCMS) has provided a means of studying the corticospinal system in a non-invasive manner in awake human subjects. Conventional use of the technique has allowed assessment of the conduction velocity and excitability of the corticospinal system in both health and disease. It has been possible to demonstrate disordered corticospinal function in conditions such as multiple sclerosis (slowing and dispersion of responses evoked by magnetic stimuli) and epilepsy (abnormal excitability).

Recently the technique of magnetic stimulation has been developed and refined to allow investigation of cortico-cortical connections. This technique relies on the use of double magnetic pulses (Kujirai *et al.*, 1993). As described earlier the first stimulus is a subthreshold conditioning shock and the second one a suprathreshold test stimulus. It is probable that the conditioning stimulus evokes both excitatory and inhibitory effects with inhibition being the dominant effect at shorter ISIs (1-6 ms). At longer ISIs (7-15 ms) excitation becomes the stronger effect.

By studying the time-course of this inhibition it is possible to obtain an indication as to the efficacy of cortico-cortical inhibitory actions. The inhibition observed when employing this technique is most probably produced at a cortical level as measures of spinal cord excitability remain unchanged (Kujirai *et al.*, 1993), and we have termed it "corticocortical inhibition". The most likely substrate for this inhibition is a network of local intracortical GABAergic inhibitory interneurones (Kujirai *et al.*, 1993).

The experiments described in this thesis examine the role of corticocortical connections in man. Much of this work was undertaken by the use of the double pulse magnetic stimulation studies and concentrates on the role of inhibitory connections. By examining corticocortical inhibition in (i) control subjects in different states (relaxed/active) and (ii) patients with a variety of movement disorders it was hoped that it would be possible to develop a better understanding of the physiology of these intrinsic cortical connections.

The next section will briefly restate some of the points made in the discussions of the experimental sections and then discuss the findings in terms of the role of intrinsic inhibitory connections within the motor cortex. Many of the ideas

suggested in this final chapter are necessarily speculative and will hopefully generate further discussion.

Effect of voluntary contraction

In the original experiments examining corticocortical inhibition Kujirai and colleagues (Kujirai *et al.*, 1993) investigated the time-course of effects when the target muscle was relaxed. The experiments described in Chapter 3 investigated the effect of voluntary contraction upon the time-course of inhibition. For these experiments (and all the others described in this thesis) a slight modification was made to the technique described by Kujirai. The intensity of the conditioning stimulus was reduced to 5% of stimulator output below the threshold for producing a response in the voluntarily activated target muscle. This resulted in conditioning stimuli that were approximately 5-10% (of stimulator output) below the intensity used by Kujirai. This modification was made to ensure that the conditioning stimulus produced no descending volley and that the inhibition seen was due to cortical, and not spinal, inhibitory processes. The results from these experiments demonstrated that there was a reduction in the amount of inhibition of the test response when the subject maintained a minimal background contraction of the target muscle. It is probable the changes seen are due to alterations in cortical inhibition and not to changes in spinal excitability. The decrease in inhibition appears to be specific to the muscle being voluntarily activated with distant relaxed muscles showing no reduction in inhibition. In fact, in the experiments examining inhibition in the FDI muscle during a contraction of the biceps muscle there is a suggestion that there might even be an increase in the inhibition. In order to be sure that this is a real effect a greater number of subjects needs to be investigated. It is interesting to speculate on the role of these changes in inhibition in the control of movement. The reduction in inhibition seen to the target muscle might represent an "opening-up" of the cortical representation to that muscle i.e. a reduction in the efficacy of inhibitory activity projecting onto the corticomotoneuronal cells projecting to the target muscle. This would allow for a rapid recruitment and modulation of the cortical population projecting to the target muscle which would facilitate accurate and rapid voluntary movements. As well as being able to recruit the appropriate output cells for an intended movement it is also necessary to

maintain inhibition of output cells that project to muscles that are unnecessary for the movement and would interfere with the intended task. If the slight increase in inhibition seen in distant muscles is real then this may be an indicator that an inhibition of "inappropriate" corticomotoneuronal cells takes place. It may be that more demanding experimental paradigms that required a greater degree of control over a variety of muscles would enhance any changes in inhibition in distant muscles.

It appears therefore as if the inhibition investigated with this technique is important for the control of normal movements. In chapters 4, 5, and 6 corticocortical inhibition was investigated in pathological conditions in which there was a disorder of movement.

Mechanism of inhibition

The mechanism behind this inhibition still remains unknown. However, as outlined in earlier chapters there is evidence to suggest that it is cortical in nature and it is known that there are many intrinsic inhibitory cells within the motor cortex. One of the surprising aspects of this inhibition is that it has such a low threshold (i.e. well below the threshold for eliciting a response in an active target muscle). When addressing the problem of what is producing this inhibition this low threshold has to be considered. One possible source of the inhibition may be the inhibitory basket cells. There are four aspects of the anatomy and physiology of these cells that suggests a role for them in the inhibition recorded with this technique (see Introduction); (i) These cells are known to make inhibitory GABAergic connections with pyramidal cells, (ii) they can be found in more superficial cortical layers (it is known that they can be found in layer III), (iii) their axons lie in a horizontal orientation (making them more likely to be excited by magnetic stimulation) and (iv) it is known that these cells have myelinated axons. Both (ii) and (iv) would help to explain the low threshold for the inhibition seen with this technique.

It is thought that the silent period seen after magnetic stimulation in voluntarily active muscle is, at least in part, dependent upon the activity of cortical inhibitory mechanisms. The question arises as to whether there is any connection between the mechanism responsible for cortico-cortical inhibition and the inhibition responsible for the silent period evoked in contracting muscle by a significantly

larger suprathreshold magnetic stimulus. In the Parkinson's disease patients this possibility was investigated by comparing the change in cortico-cortical inhibition with the change in silent period duration and no correlation was seen. The results did show that on average when the patients were off their medication the silent period duration was shorter than controls and that there was a reduction in the level of cortico-cortical inhibition. It may be therefore that the two forms of inhibition are linked in some way. There was no clear correlation in any individual and this lack of correlation may be due to a number of factors. One possibility is that the silent period is measured during voluntary contraction and it has been shown that activity alters the level of corticocortical inhibition. Therefore it would be more appropriate to look for correlation's during voluntary contraction. As discussed in Chapter 4 there are differences in the duration and depth of inhibition in the two cases when comparing the corticocortical inhibition in relaxed muscle with the silent period. However, these differences are reduced when the corticocortical inhibition is recorded during activity. It therefore seems possible that the two forms of inhibition share, at least some, common mechanisms.

Parkinson's disease

Using the techniques described a group of patients suffering from Parkinson's disease (PD) were investigated. Patients with PD present with a variety of abnormal motor signs. As one of the major targets for the basal ganglia is the primary motor cortex is was reasonable to examine cortical inhibitory function in this group to look for disturbances in inhibition that might play a part in their disabling movement disorders. This group of patients was examined at rest as this is the condition in which the inhibition is greatest. As the experiments in Chapter 4 report there was an abnormality of inhibition in this group, with a reduction in inhibition being seen at short ISIs. This reduction appeared to be more prominent at certain ISIs.

It appears, therefore, as if deranged basal ganglia function can lead to alterations in the efficacy of intrinsic inhibitory actions in the primary motor cortex. Little is known about the mechanism which brings this change about. However, it is known that in MPTP treated monkeys (which form a good working model for PD) cortical cells in the motor cortex become less selective in their firing patterns

(Doudet *et al.*, 1990). Similar changes in firing patterns are seen after the local administration of the GABA antagonist bicuculline (Matsumura, Sawaguchi and Kubota, 1992). Therefore, it may be that altered basal ganglia function can modify the efficacy of intrinsic GABAergic connections. It is possible that a reduction in cortical inhibition is responsible for some of the symptoms and electrophysiological findings in PD. If there is a reduction in the level of cortical inhibition it might be expected that a given afferent input might produce an exaggerated output. It is possible that this may be the mechanism behind the known enhancement of the long-latency stretch reflex seen in PD. One of the most disturbing symptoms of PD is the ON dose dyskinesias. These often become greatly disabling after many years of treatment with L-Dopa. L-Dopa tends to "normalise" the output from the disorded basal ganglia and it may be that a disinhibited motor cortex on receipt of a normalised input from this region responds with an excessive and inappropriate response i.e. excessive movement in the form of dyskinesias. It is interesting to speculate as to whether the severity of the dyskinesias might be reduced by treatment with a GABA agonist such as vigabatrin.

Focal dystonia

In Chapter 5 experiments in patients with focal task specific dystonia are described. Most of these patients suffered from Writer's cramp. In cases where any pathology has been demonstrable it was found within the putamen, caudate, thalamus and globus pallidus or their connecting pathways (Rothwell and Obeso, 1987). Again it was reasonable to investigate whether in this group of patients there might be a disturbance of cortical inhibitory function.

As described in the results there was a reduction in inhibition at short ISIs. However, the findings were slightly different from those seen in the Parkinsonian patients. The dystonic patients had a more widespread lack of inhibition with no particular ISI exhibiting a striking lack of inhibition as was seen in the PD patients. The reason for this remains unclear and further investigation is needed with studies of more subjects. Again it is possible to propose a model in which reductions in corticocortical inhibition could lead to some of the symptoms of focal dystonia. If there is a lack of intrinsic inhibition within the primary motor cortex then an instruction to this region to move might produce recruitment of corticomotoneuronal

cells that project to muscles unnecessary for the intended movement. Again (as suggested above for the PD patients) it might be interesting to investigate the effect of a selective GABA agonist on the symptoms of focal dystonia.

It appears as if there may be subclinical abnormalities of cortical inhibition in many of the patients with focal dystonia. There were abnormalities of inhibition in the hemisphere contralateral to their "unaffected" hand. However, many patients with this type of dystonia go on to develop symptoms in their non-dominant had if they try to adopt to using this hand for tasks previously performed with their affected hand. It may be that the abnormalities in inhibition are present from early on in the condition and only manifest once the hand becomes much more active. One possible trigger for the appearance of symptoms is the increased afferent input to the motor cortex.

It would be interesting to investigate patients with more generalised dsytonia to determine whether there are similar abnormalities when more proximal muscles are tested. However, testing more proximal muscles presents certain problems in many subjects. The major problem being that in many subjects the threshold for evoking responses in proximal muscles is far higher than that for evoking responses in distal muscles.

Cortical myoclonus

Cortical myoclonus is a relatively rare disease and it is difficult to get sufficient numbers of patients to organise a comprehensive study. The experiments described in Chapter 6 describe the findings in a group of cortical myoclonics studied over a three year period at the National Hospital. Some of the experimental results were obtained from small numbers but as stated it is very difficult to find suitable patients. Another complicating factor is the medication taken by many of these patients makes interpretation of some of the data difficult. For example, it is well known that these types of medication can alter thresholds to magnetic stimulation (Hufnagel *et al.*, 1990; Reutens *et al.*, 1993). However, even these limited results provide some insight into the role of inhibition in this challenging condition.

The patients studied in these experiments were divided into two groups; *spreaders* and *non-spreaders*. This division was made on the basis of the number of

muscle jerks that appeared to involve spread of abnormal activity within the motor cortex or between hemispheres (see Chapter 6 for full explanation). There was a marked reduction in the corticocortical inhibition in the *spreaders*, as well as a similarly striking lack of transcallosal inhibition. The reduction in corticocortical inhibition in this group of patients was greater than that seen in other patient groups studied in this thesis. For example, if the average level of inhibition across the normally inhibited ISIs (1-6 ms) is compared in the *spreaders*, dystonics and normals it can be seen that the *spreaders* are the most abnormal (*spreaders* 107±23% of control; dystonic patients 80±17%; controls 50±15%). It is possible that the severity of the abnormalities in myoclonus reflects the intrinsic pathology in the cortex.

These findings are consistent with an abnormality of inhibition within and between regions of motor cortex being responsible for the spread of abnormal cortical activity leading to multifocal time-locked muscle jerks. The *non-spreaders* had some minor reduction in corticocortical inhibition but this reduction proved to be non-significant. The level of transcallosal inhibition was the same as in the control subjects. It was argued that these findings were consistent with the confinement of abnormal myoclonic discharges to the region of cortex from which they originated.

A number of the patients suffered from seizures of varying frequency and it is interesting to note that there appeared to be no correlation between seizure frequency and the level of inhibition. Indeed, several of the patients had a complete lack of recordable corticocortical or transcallosal inhibition and yet were still seizure free. It appears then as if the reduced corticocortical inhibition seen in these patients may be an epileptic epiphenomena and that it is much more closely associated with the presence and extent of myoclonus. However, it has previously been reported that there are abnormalities in inhibition in patients with focal epilepsy (Fong *et al.*, 1993). Therefore, the relationship between cortico-cortical inhibition and epilepsy remains unclear and requires further work.

Using this technique it is possible to demonstrate abnormalities of inhibition in three different groups of patients. The pathology in these groups is either in the basal ganglia (which has an important input to the primary motor cortex) or within

the motor cortex itself. At first sight, it is perhaps slightly disappointing that these different conditions all result in abnormalities of inhibition. However, on closer inspection of the results it can be seen that there are subtle differences in the abnormalities of inhibition in the three different diseases. Therefore, it is possible that these subtle differences reflect the variety of clinical problems seen in these conditions.

Deafferentation

In the final experimental chapter (Chapter 7) the effect of deafferentation has been investigated. There have been extensive reports of changes in cortical organisation that take place following deafferentation in both animals and humans.

The experiments described in this chapter were initiated because it may be that the corticocortical inhibition recorded using magnetic stimulation plays a role in the maintenance of cortical representations. As discussed in the Introduction it is probable that cortical representations are maintained, at least in part, by the action of local GABAergic inhibitory neurones. It is also thought that the inhibition recorded following magnetic conditioning stimuli is due to activity in intrinsic inhibitory circuits. It is possible therefore that alterations in motor cortical organisation and corticocortical inhibition (as measured by the double pulse magnetic stimulation technique) are brought about by action of the same set of neurons.

Firstly, it was decided to attempt to replicate the findings of other groups in traumatic amputees and normals with ischaemic blocks of the hand. Using the techniques of cortical mapping and intensity curve recording it was shown, in agreement with the previous reports, that the "size" of a cortical representation of a muscle proximal to the amputation or ischaemic block was increased when compared to the control. In practice this meant that the responses were larger and could be recorded following stimulation at more scalp sites. As discussed in Chapter 7 it is impossible to be sure as to whether this truly represents an expansion of the cortical representation or just an increase in excitability of a constant representation.

As in previous groups experiments these recordings were made while the target muscle was relaxed. However, surprisingly, when the subject maintained a minimal contraction of the target muscle during recording these changes were not apparent. This unexpected finding questioned the physiological importance of the

apparent. This unexpected finding questioned the physiological importance of the alterations of cortical excitability seen when the target muscle was relaxed. It is proposed that in the relaxed state the motor cortex is under the influence of a tonic inhibitory input. This maintains relaxation and stops unwanted movements. During a voluntary movement the tonic input is reduced in cortical regions projecting to muscles needed for the intended movement therefore allowing efficient recruitment of the projection. In the case of amputation or ischaemic block a group of muscles becomes "unavailable for selection". There is therefore little point in maintaining a tonic inhibitory influence on the corticomotoneuronal cells projecting to the "unavailable" muscles. This would result in the cortex being of similar excitability in both the relaxed and active conditions. It therefore appears as if the change in cortical excitability seen in the relaxed condition following deafferentation (either temporary or permanent) is an epiphenomenon and has little physiological importance in the active condition. It would be interesting to test this hypothesis by making further measurements in these groups of subjects. For example, measurements of reaction time or the speed of force generation could be made in muscles proximal to the block/amputation to investigate if there was any measurable advantage when compared to the control muscle.

It was intended to use the technique of corticocortical inhibition to investigate changes in inhibition in these groups of subjects. However, there are several problems. Firstly, there is a limited time available when using ischaemic blocks and the technique of corticocortical inhibition requires some time to be comprehensively investigated. The second problem is that it is difficult to do repeated investigations on control subjects with ischaemic blocks. As reported in Chapter 7 the changes in cortical excitability reported tend to be much less obvious on successive occasions. Finally, recordings in amputees were made from muscles that are more proximal than the small hand muscles normally investigated with this technique. Although it is possible to record inhibition in more proximal muscles in some subjects, because of the higher threshold for stimulation, it proves very difficult in the majority of subjects. It has therefore proved to be very difficult to investigate this phenomenon using the technique of double pulse magnetic stimulation. In the future it may be possible to overcome the final problem by the use

of newly developed multiple pulse stimulators that can provide higher pulse intensities. Also, by performing selective nerve blocks, it may be possible to study these changes in hand muscles in naive subjects.

Motor threshold and corticocortical inhibition

One interesting point that can be made from the experiments described is the lack of relationship between the threshold for evoking muscle responses with a magnetic stimulus and the level of corticocortical inhibition. This is the case in both the normals and patient groups. In all the patient groups studied there was no correlation between threshold and corticocortical inhibition. This is also true when looking at patients who were receiving no medication. Strikingly, several of the myoclonic patients who were on no medication had high thresholds in the presence of a complete lack of inhibition. It is probable that the threshold for stimulation is dependent upon the balance of inhibition and excitation being exerted on the corticospinal neurons. Changes in either inhibition or excitation would have an effect on the threshold. The time-course of corticocortical inhibition is also probably dependent upon the balance of inhibition and excitation but at the shorter ISIs inhibitory inputs are likely to be more influential. Changes in facilitatory input may well affect threshold while having relatively little impact on the level of inhibition. This technique of double pulse magnetic stimulation tests the *excitability*, and not the on-going activity, of this inhibitory system. These two parameters may not necessarily be the same.

The role of corticocortical inhibition

From data presented in this thesis and previous work (Kujirai *et al.*, 1993) it is likely that corticocortical inhibition reflects the activity in intrinsic inhibitory interneurones within the motor cortex. There are several findings that suggest that these inhibitory interneurones might utilise GABA as their neurotransmitter. Firstly, GABA is by far the most common inhibitory neurotransmitter found in cells within the motor cortex. Secondly, from some preliminary experiments it appears as if modulation of the level of GABA within the motor cortex by the use of selective GABA agonists can provoke alterations in the level of corticocortical inhibition (authors unpublished data).

If it proves to be the case that the inhibition recorded with this technique does reflect activity in GABAergic interneurons then the role for this inhibition may become clearer. It is known from animal experiments that alterations in GABA levels leads to a loss of specificity of cortical cells. Also, it is known that lesions in the basal ganglia following MPTP administration in monkeys leads to a loss of directional specificity in cortical cells. It is therefore possible that a change in the basal ganglia output modulates the tonic activity in a set of GABAergic inhibitory interneurones in the primary motor cortex. As the results in Chapter 4 demonstrate there are abnormalities of corticocortical inhibition in PD and this may reflect alterations in the tonic set of the inhibitory interneurones.

It appears as if this group of corticocortical inhibitory cells might have an important role to play in "setting" the cortex in preparation for voluntary movement. Modulation of this inhibition may facilitate the recruitment of muscles needed for the intended movement while also inhibiting muscles inappropriate for the movement. A deficiency in this inhibition would then be expected to lead to excessive and inappropriate movement as is seen in the movement disorders studied in this thesis.

Further experiments

All the conclusions drawn in this thesis rely on the idea that this technique of double pulse magnetic stimulation is monitoring the activity in inhibitory neurons within the motor cortex. As discussed in this thesis we have evidence to suggest that this is the case. However, there are further experiments that would be useful in further confirming this hypothesis.

It would be most interesting to record the descending volleys evoked by paired magnetic stimuli in the spinal cord or at a more rostral site. It would be informative to know which of the components of the volley were inhibited by the conditioning stimulus. It is surprising that the conditioning stimulus can have a relatively powerful effect at such low intensities. However, there are groups of inhibitory cells within the more superficial cortical layers that lie in an orientation that makes them susceptible to the electric currents induced by magnetic stimuli (see Introduction). By examining the effect of conditioning stimuli upon the components of the descending volley it may be possible to infer more information regarding the

neurons that are activated by the conditioning stimulus. Traditionally it has only been possible to record descending volleys in the spinal cord when patients are anaesthetized. As magnetic stimulation is very susceptible to anaesthetic agents this type of investigation has proved very difficult. However, it may be possible in the near future to record descending volleys from in-dwelling leads in the cervical region in conscious subjects.

Finally, this technique may offer a means of monitoring improvements in patients who undergo surgical treatment of conditions with associated movement disorders. For example, it is important to document the change in patients conditions following pallidotomy for the treatment of PD. This involves a wide range of imaging, pyschological, and electrophysiological tests. Recording of corticocortical inhibition pre and post surgery might provide useful information about the condition of the primary motor cortex. Also, investigations of this type might further elucidate the physiology responsible for the inhibition.

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Appendix 1

Webster Rating Scale (Webster, 1968)

Bradykinesia

0 No involvement
1 Detectable slowing of the supination-pronation rate evidenced by beginning to have difficulty in handling tools, buttoning clothes and with handwriting.
2 Moderate slowing of supination-pronation rate, one or both sides, evidenced by moderate impairment of hand function. Handwriting is greatly impaired, micrgraphia present.
3 Severe slowing od supination-pronation rate. Unable to write or button clothes. Marked difficulty in handling utensils.

Rigidity

0 Non-detectable
1 Detectable rigidity in neck and shoulders. Activation phenomenon is present. One or both arms show mild, negative resting rigidity.
2 Moderate rigidity in neck and shoulders. Resting rigidity is positive when patient not on medication.
3 Severe rigidity in neck and shoulders. Resting rigidity cannot be reversed by medication.

Posture

0 Normal posture. Head flexed forward less than 4 inches (10cm).
1 Beginning poker spine. Head flexed forward upto 5 inches (12cm).
2 Beginning arm flexion. Head flexed forward upto 6 inches (15cm). One or both arms raised but still below waist.
3 Onset of simian posture. Head flexed forward more than 6 inches (15cm). One or both arms raised above waist. Sharp flexion of hand, beginning interphalangeal extension. Beginning flexion of knees.

Upper extremity swing

0 Swings both arms well.
1 One arm definitely decreased in amount of swing.
2 One arm fails to swing.
3 Both arms fail to swing.

Gait

0 Steps out well with 18-30 inch stride (45-75cm). Turns about effortlessly
1 Gait shortened to 12-18 inch stride (30-45cm). Beginning to strike one heel. Turn around time slowing. Requires several steps.
2 Stride moderately shortened - now 6-12 inches (15-30cm). Both heels beginning to strike floor forcefully.
3 Onset of suffling gate, steps less than 3 inches (8cm). Occasional stuttering- type or blocking gait. Walks on toes - turns around very slowly.

Tremor

0 No detectable tremor found.
1 Less than 1 inch (2cm) of peak-to-peak tremor movement observed in limbs or head at rest or in either hand while walking or during finger to nose testing.
2 Maximum tremor envelope fails to exceed 4 inches (10cm). Tremor is severe but not constant and patient retains some control of hands.

3 Tremor envelope exceeds 4 inches (10cm). Tremor is constant and severe. Patient cannot get free of tremor while awake unless it is pure cerebellar type. Writing and feeding self are impossible.

Facies

0 Normal. Full animation. No stare.
1 Detectable immobility. Mouth remains closed. Beginning to display features of anxiety or depression.
2 Moderate immobility. Emotion breaks through at markedly increased threshold. Lips parted some of the time. Moderate appearence of anxiety or depression. Drooling may be present.
3 Frozen facies. Mouth open 0.25 inch (60mm) or more. Drooling may be severe.

Seborrhea

0 None
1 Increased perspiration, secretion remaining thin.
2 Obvious oiliness present- secretion much thicker.
3 Marked seborrhea, entire face and head covered by thick secretion.

Speech

0 Clear, resonant, easily understood.
1 Beginning of hoarseness with loss of inflection and resonance. Good volume and still easily understood.
2 Moderate hoarseness and weakness. Constant monotone, unvaried pitch. Beginning of dysarthria, hesitancy, stuttering, difficult to understand.
3 Marked harshness and weakness. Very difficult to hear and understand.

Self-care

0 No impairment]
1 Still provides full self-care, but rate of dressing definitely impeded. Able to live alone and often still employable.
2 Requires help in certain critical areas, such as turning in bed, rising from chairs etc. Very slow in performing most activities but manages by taking much time.
3 Continuously disabled. Unable to dress, feed self, or walk alone.